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Official Organ of The American Phytopathological Society

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FRED CARLETON STEWART

1868–1946

HARRY M. FITZPATRICK[1]

Fred Carleton Stewart, for many years botanist of the New York Agricultural Experiment Station at Geneva, died April 24, 1946, ten years after retirement from active service. In his long and productive career he made his chief contribution in research in the field of plant pathology. His death marks the passing of another of the older generation of distinguished workers who pioneered in the study of plant diseases in America.

Professor Stewart was born, February 13, 1868, at French Creek, New York, a small village in Chautauqua County in the extreme southwestern corner of the State. He was the only child of Almeron Lyman Stewart (1842–1931) and Charlotte Eliza Hubbard (1842–1920). His father was a farmer, and his own boyhood was spent in rural surroundings. When he was still a small child his parents left the French Creek neighborhood and moved to Iowa where they settled on a small farm in Adair County near Greenfield. There he attended the public schools and, in 1888, graduated from the Adair County Normal Institute. As he held teachers' certificates from 1886 to 1890, he probably taught for a time in the local schools. He graduated from Iowa State College in 1892 with the degree of Bachelor of Science, and in 1894 received the degree of Master of Science from the same institution. He was Assistant Botanist of the Iowa Agricultural Experiment Station from 1891 to 1894. At that period he published nearly a dozen short papers, several of them in co-authorship with Professor L. H. Pammel, Botanist of the Station. They indicate that the training received under Pammel was very important in his early development, and they reveal his growing interest in plant diseases and parasitic fungi. One of his fellow students in the laboratory was George Washington Carver, the talented negro, who later was prominent for many years at Tuskegee Institute in Alabama. Stewart and he carried on some of their investigations together and, when Stewart left Iowa, Carver succeeded him as Assistant Botanist. They published a single paper jointly, reporting results obtained in inoculation experiments with a species of *Gymnosporangium*.

In December, 1894, the Board of Control of the New York Agricultural Experiment Station at Geneva called Stewart from Iowa to the position of Mycologist at the newly created Long Island Branch Station at Jamaica. From the beginning, it was understood that he was to concern himself chiefly with plant disease control. Arriving at Jamaica, he found only an improvised laboratory, located in a crowded tenement house. With meagre equipment under trying conditions, he began the career in his native State that

[1] The writer is under obligation to members of Professor Stewart's family who provided facts concerning his early life. Expression of appreciation is due also to colleagues at Ithaca and Geneva who aided in evaluating his work, and approved the finished manuscript.

FRED CARLETON STEWART
1868–1946

was to extend over four decades. With characteristic industry and determination he soon had field experiments outlined and under way. Since in his later years he was known especially for his long-continued and diversified researches on the diseases of potatoes and their control, it is noteworthy that his first season's work on Long Island definitely established his interest in the problems of potato growing. His first bulletin on the spraying of potatoes, covering the results of his field experiments in 1896, appeared only fifteen years after the discovery of Bordeaux mixture. In those early days much remained to be determined concerning the efficacy of spray mixtures on different plants and methods of application. Stewart's other publications at that period dealt with diseases of carnations, cucumbers, and other market-garden crops. His most noteworthy accomplishment while at Jamaica was perhaps his demonstration of the bacterial nature of a newly discovered wilt disease of sweet corn, then prevalent on Long Island. His paper on the disease, including a detailed description of the cultural characters of the causal organism, appeared in 1897 when as yet only a few species of bacteria had been shown conclusively to be plant pathogens. The following year Erwin F. Smith verified his observations, called the malady "Stewart's disease of sweet corn," and named the organism *Pseudomonas stewarti.* Also, in 1899, Stewart described and figured a bacterial disease of onions without naming the organism involved. In this case many years later Burkholder, in establishing *Phytomonas alliicola,* expressed the conviction that Stewart had dealt with the same species and apparently had been the first to recognize the disease.

Stewart remained at Jamaica only three years. Then, convinced of his need for additional phytopathological training, he obtained a year's leave of absence and entered the Graduate School of Cornell University. After a very brief stay in Ithaca he decided that recent advances in techniques could best be learned in some of the European laboratories, and in the winter of 1897–1898 he spent several months in travel and study abroad. For a time he was in residence at the University of Munich in contact with Robert Hartig and Karl von Tubeuf. At the end of his first year on Long Island he had returned to Iowa on vacation, and at Bassett in Chickasaw County, November 20, 1895, had married Annie Alene Chestek, daughter of John and Amelia (Cummings) Chestek. She accompanied her husband on the trip to Europe. It was an outstandingly pleasant and profitable experience for both of them.

On returning to America, Stewart was appointed Botanist and Head of the Department of Botany of the New York Agricultural Experiment Station. He entered on his new duties at Geneva in August, 1898. In the Annual Report of the Station for 1897, Director W. H. Jordan had stated that the Botanist would "be selected with reference to his fitness to take up investigations in plant pathology." Though, in the years immediately preceding Stewart's appointment, miscellaneous matters of phytopathological nature had been handled by the Horticulturist, the large fruit and vegetable

growing interests of the State were demanding that more attention be given to plant disease control. In earlier days, soon after the Station was established, J. C. Arthur had been appointed its first Botanist, but remained at Geneva only three years, leaving in 1887 to go to Purdue University. After a lapse of eleven years the position was now revived for Stewart. It was not until 1920 that, with reorganization of the administrative work of the Station, the Department of Botany was renamed the Division of Botany, and Stewart's title became Chief in Research. Finally, in 1936, only a few weeks before his retirement from active service, the Division of Botany became the Division of Plant Pathology. In 1923, with the merging of the Station and the New York State College of Agriculture at Ithaca he had become also Professor of Botany in Cornell University, and at his retirement was made Professor Emeritus. In addition to his three years as Mycologist at the Jamaica branch station, he served the New York Agricultural Experiment Station at Geneva for thirty-eight years. At his death in 1946, at the age of seventy-eight, his years of residence in Geneva lacked only two of covering a half century.

Stewart published regularly and frequently, chiefly in the field of plant pathology. His papers totaled more than one hundred and sixty, and included more than seventy-five Station bulletins and reports. In 1902 he began an extensive series of experiments on potato spraying, and annually for ten years published a bulletin reporting his results and conclusions. In 1912, in Bulletin 349, he summarized the work for the ten-year period. This decade of experimentation on potato disease control is one of the classics of American phytopathology. Stewart became interested early in the obscure maladies of potatoes which are now known as the virus diseases. He studied their characteristic symptomatology, sought to separate them into mosaic, leaf roll, spindling sprout, and other such categories, and attempted their control by isolation of the seed plot and by roguing. He carried on many miscellaneous experiments in potato culture, and developed methods of potato disease control that became standard practices. Keeping in contact with work in progress at other stations, he shared his ideas freely with other investigators. In the early years he cooperated with Dr. L. R. Jones who at that period was also deeply interested in potato diseases in Vermont.

Though Stewart was probably best known for his long-continued and diversified studies on potatoes, he published numerous papers on diseases of many other plants. He had a predilection for assembling short notes on relatively unrelated subjects within the covers of a single bulletin. Hidden away in such bulletins will often be found valuable early records. He was an exceptionally keen observer, and was most methodical in keeping detailed notes on his field observations and activities. Each morning on reaching his office it was his routine procedure to write down carefully records of the preceding day. These notes on small uniform-sized sheets of paper were filed by him in chronological sequence for each disease. His handwriting was very attractive and wholly legible, and the entire set of notes, to which he gave meticulous care, is preserved at Geneva available for consultation.

During Stewart's long period of service at the New York Agricultural Experiment Station he made a distinguished and enviable record. He was a successful administrator, highly respected and well-liked by his associates, and his Department was outstandingly productive in research. His subordinates shared with him in its publications and at all times received full credit for their contributions. His viewpoint as a plant pathologist was definitely practical. He placed major emphasis on plant disease control, and sought earnestly to render real service to the grower. In consequence he gained popular and official support for his work, even in the early days when the economic significance of the investigation of plant diseases was not as yet generally conceded.

Professor Stewart's eminence in the field of plant pathology was given recognition by the American Phytopathological Society in its early years when it elected him its fifth President. He was a charter member of the Society and, though not especially active in its affairs, was deeply interested in all phytopathological matters. He also became a charter member of the Mycological Society of America and was elected a Fellow of the American Association for the Advancement of Science.

In 1908, when Stewart was forty, he purchased a cottage camp on Seventh Lake in the southwestern Adirondack Mountains, and throughout the following thirty-two years spent a portion of every summer there vacationing with his wife and children. The lake is circled by low mountains and closely bordered by dense forest that stretches away unbroken for miles. The camp, named by the Stewarts The Birches, stands in a secluded spot on the far shore remote from the highway, and provided a quiet retreat to which Stewart welcomed his botanical friends. In 1915, he was visited by Professor George F. Atkinson, who at that period was actively engaged in the taxonomic study of the Agaricaceae. In a ten-day period they collected and studied more than two hundred species in the surrounding woods. Atkinson returned there in the summers of 1916 and 1917, and, stimulated by his enthusiasm, Stewart's own interest in the higher Basidiomycetes was definitely aroused. He kept a list of the species identified by Atkinson, and in the following years added to it, having in mind the publication of an inclusive list of the prominent fungi of the Seventh Lake region. In 1921, Doctor Calvin H. Kauffman spent three weeks with him there busily collecting and identifying, and in 1931 the Stewarts entertained a group of well-known American mycologists who assembled to collect Agaricaceae with Doctor Jakob E. Lange of Denmark. In 1934, the second annual summer meeting of the Mycological Society of America was held at Seventh Lake with headquarters established at the Stewart camp. About forty-five mycologists and plant pathologists attended, and the Stewarts labored prodigiously to make the meeting outstandingly pleasant and successful. In other years Stewart collected alone or in the company of students of the fungi who occasionally visited him. His carefully edited list of Seventh Lake Fungi has not been published, but has been preserved in typewritten form in connection with his

herbarium. It includes about 750 species in 120 genera, most of them being Hymenomycetes. The list constitutes a noteworthy enumeration of the prominent fungi of the mixed hardwood and coniferous forest typical of northern New York. Stewart's growing interest in the mushrooms in his later years led him to publish several bulletins on a few easily recognized edible species. He also wrote a popular circular entitled "How to know the mushrooms and toadstools."

Following his retirement in 1936 Stewart spent several winters in Florida collecting leisurely and seeking rest and relaxation. The loss of his wife in the autumn of 1937 was a bitter blow and was followed by other misfortunes. The property on Seventh Lake was sold in 1941, and during his remaining years he resided at Geneva with the family of a daughter. The news of his death came as a shock to many of his old friends who had failed to keep in touch with him in his last days.

Professor Stewart was a tall slender man of erect carriage and natural dignity of manner that seemed at times to verge on austerity.[2] At heart he was a kindly person taking sincere pleasure in being helpful to others. He had a pleasant disposition, a well developed sense of humor, and thoroughly enjoyed a good joke, especially when it dealt with human interest or reaction. He was a most thoughtful host and an intensely loyal friend. The personification of trustworthiness, he was absolutely honest in thought and deed. An especially modest man, he avoided the appearance of seeking honors or distinction, and gladly made way for more aggressive contemporaries. There was never any doubt, however, as to his position on a question, and he was unwavering in the defense of his convictions. He was by temperament distinctly conservative. Premature or ill-considered actions were distasteful to him. Unsound or doubtful propaganda, designed to obtain financial backing for plant disease work lacked his support. For example, at the historic chestnut blight conference at Harrisburg, when the spread of the disease to the Appalachian forests threatened catastrophe, he opposed seeking a heavy appropriation for a spectacular program which he regarded as of doubtful expediency. He possessed too a streak of obstinacy, which found him at times supporting a lost cause long after there was any remaining hope of success. This was true of his advocacy of simplified spelling which he used in correspondence for a considerable period of years and even employed in a manuscript submitted by him as a test case to the editor of Phytopathology. He served for several years on the Advisory Council of the National Simplified Spelling Board. In all such cases, whether one agreed with him or not, there could be no question of his sincerity. One of his most noticeable characteristics was his earnestness in the detailed prosecution of any task that he undertook, even though it were relatively unimportant. His attitude toward his work was intensely serious, and steadfastness of purpose marked his days.

[2] Photograph: Mycologia 28: 99. 1936.

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MISCELLANEOUS GREENHOUSE TESTS WITH VARIOUS SOIL FUMIGANTS FOR THE CONTROL OF FUNGI AND NEMATODES

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Several years of experimental work and field trials are usually required to establish the merits of a new soil fumigant. However, by means of greenhouse tests it has been possible to evaluate new soil fumigants accurately in a relatively short time. This paper deals with the results obtained with various fumigants for the control of fungi and nematodes in certain greenhouse tests.

FUNGICIDAL EFFICACIES OF VARIOUS FUMIGANTS

The fungicidal efficacies of various fumigants were compared as follows. A quantity of sandy loam soil was infested with a species of *Fusarium* capable of causing damping-off of peas. The soil was thoroughly mixed and placed in one-gallon glazed crocks (6½ inches diameter, 7 inches deep). The crocks were treated by placing the desired quantity of the fumigant in a hole 3½ to 4 inches deep in the center of the crock. The hole was then closed with soil and the surface "sealed" with 100 ml. water. In one treatment formalin was also applied as a water drench mixed throughout the soil. Each treatment was replicated 4 times. Eight to fourteen days after treatment each crock was planted with 50 pea seeds (var. Thomas Laxton) by removing a 2-inch layer of soil, distributing the seeds in the crock, and replacing the soil. Proper precautions were taken to prevent contamination of the soil during this operation. The soils were then maintained at a high moisture level to permit germination of the seed and favor the occurrence of damping-off. Preliminary counts of the stands of pea seedlings were made about ten days after planting, and final counts after about 20 days. A summary of the data on final stands of pea seedlings in three experiments is presented in table 1.

Chloropicrin was by far the most fungicidal of the fumigants tested. The 10 per cent methyl bromide solutions and the DD mixture (dichloropropylene and dichloropropane) had some fungicidal effect, but at dosages sufficient for nematode control, about 1.5 ml. and 0.5 ml., respectively (Table 2), they are not effective fungicides. When compared on this basis, DD mixture would be expected to have greater fungicidal effect than the methyl bromide solutions. It made no difference whether the methyl bromide was carried in ethylene dichloride or xylene. The formaldehyde drench gave good control of damping-off, but this chemical was not effective when injected, because its high solubility in water prevents an even distribution of

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TABLE 1.—*Efficacies of various soil fumigants for controlling Fusarium in the soil*

Fumigant	Dosage[a] (ml.)	Average stand of peas		
		Exp. 1 Per cent	Exp. 2 Per cent	Exp. 3 Per cent
Check	.....	2.0	8.0	17.2
Steam	.....	96.0	91.5	98.0
Chloropicrin	2.00	94.0**	.....	.....
	1.00	95.0**	.....	.....
	0.50	94.5**	95.0**	87.0**
	0.25	.....	85.0**	66.0**
	0.10	.....	94.0**	84.0**
DD Mixture (crude)	4.00	.....	.....	79.6
	3.00	.....	.....	63.6
	2.00	58.5**	.....	51.6
	1.00	50.5**	.....	.....
	0.50	37.5**	.....	.....
Dowfume G[c]	10.00	.....	83.5**	50.4**
	7.50	.....	77.0**	81.6**
	5.00	32.5**	73.0**	75.2**
	2.50	45.5**	.....	.....
	1.00	1.5	.....	.....
Methyl bromide-xylene (1–9)	10.00	.....	92.0**	62.0**
	7.50	.....	83.3**	62.4**
	5.00	29.5**	72.5**	28.8**
	2.50	50.4**	.....	.....
	1.00	5.0	.....	.....
Formalin (injection)	8.00	.....	40.5**	.....
(drench)	8.00	.....	96.0**	.....
(injection)	4.00	.....	27.0	.....
(drench)	4.00	.....	89.5**	.....
Carbon bisulfide	8.00	.....	.....	15.2
	6.00	.....	.....	6.4
	4.00	.....	38.5*	.....
	2.00	.....	7.0	.....
Tetrachlorethane	6.00	.....	.....	23.6[b]
	5.00	.....	82.6	33.6*
	4.00	.....	.....	31.2
	2.50	.....	37.5*	.....
Ethylene dichloride	6.00	2.0	.....	.....
	4.00	2.0	.....	.....
	2.00	3.5	.....	.....
Ethylene dibromide	0.50	.....	4.0[b]	.....
	0.25	.....	13.5	.....
Xylene	6.00	3.5	.....	.....
	4.00	2.0	.....	.....
	2.00	0.0	.....	.....
Toluene	8.00	.....	22.0	.....
	4.00	.....	17.0	.....
Dichlorethyl ether	1.50	.....	.....	2.0
	2.40	.....	.....	0.0
Dichlorisopropyl ether	2.50	.....	.....	0.0
Least difference required for significance	19: 1	13.96	24.04	14.76
	99: 1	18.64	31.92	19.56

[a] Dosage used per 1-gal. crock of soil.
[b] Injury to seeds from residual fumigant.
[c] 10 per cent methyl bromide, 67 per cent ethylene dichloride, and 23 per cent carbon tetrachloride by volume.
* Odds of 19: 1—better than check.
** Odds of 99: 1—better than check.

its vapor throughout the soil. The other fumigants were not effective at the dosages tested.

Observations in these and in other experiments on the numbers of weeds appearing in crocks treated with the various fumigants indicate that there is a high correlation between the fungicidal efficacy of fumigants and their ability to kill the seeds of higher plants. There is no apparent relationship, however, between the period of time that a phytotoxic concentration of a fumigant persists in the soil and its ability to kill weed seeds.

THE NEMATOCIDAL EFFICACIES OF VARIOUS FUMIGANTS

The nematocidal efficacies of various fumigants were compared as follows. A quantity of sandy loam soil was infested with macerated root-knot nematode-infested roots. This soil was thoroughly mixed, placed in 1-gallon crocks, and treated as previously described. Each treatment was replicated four times. About ten days after treatment tomato seedlings or squash seeds were planted in each crock. After these plants had grown for about 4 weeks they were carefully removed from the soil and the number of nema-

TABLE 2.—*Efficacies of various soil fumigants for controlling the root knot nematode in the soil*

Fumigant	Dosage[a] (ml.)	Average number of nematode galls on indicator plants			
		Exp. 1	Exp. 2	Exp. 3	Exp. 4
Check	.....	158.8	65.7	177.3	137
Ethylene dibromide	1.00	0	.....	0.0	.....
	0.75	.....	.....	.....	0
	0.50	.....	.....	0.0	.....
	0.25	.....	.....	.....	0
Crude DD Mixture	1.50	0.0	4.0	.....	.....
	1.00	1.0	0.3	.....	1
Redistilled DD Mixture	1.50	0.0	0.9	.....	.....
	1.00	16.8	8.3	.....	.....
Tetrachlorethane	4.00	.....	.....	0.3	.....
	2.00	.....	.....	0.3	.....
Dowfume G	3.00	1.2	5.1	0.3	.....
	2.50	.....	.....	.....	2
Dichlorisopropyl ether	2.00[b]	5.8	0.0	.....	.....
	1.50[b]	4.0	0.0	.....	.....
Dichlorethyl ether	1.00[b]	0.0	0.0	.....	.....
	0.50[b]	0.0	0.9	.....	.....
	0.27	.....	.....	.....	34
Xylene	7.00	.....	.....	0.0	.....
	3.50	.....	.....	0.0	.....
Ethylene dichloride	6.00	.....	.....	.....	2
	5.00	.....	.....	0.3	.....
1,1 Dichloroethane	5.00	.....	.....	1.3	.....
	3.00	.....	.....	88.3	.....
Propylene dichloride	12.00	.....	.....	.....	40
Chloropierin	1.00	143.4	57.6	.....	293

[a] Dosage used per 1-gal. crock of soil.
[b] Toxic to plants. Impossible to get plants to grow.

tode galls on their roots counted. The data obtained from four such experiments are summarized in table 2.

The number of galls developing on the roots of these indicator plants is a good measure of the effectiveness of the fumigants. It should be noted, however, that when soil is very heavily infested with nematodes, plants growing in it are usually stunted and it is probable that whenever a plant is stunted, there is a reduction in the number of galls on the roots. This may give misleading indications of the effectiveness of some of the poorer treatments and also the amount of infestation of the soil as indicated by the numbers of galls on the check plants.

Ethylene dibromide was the most powerful nematocide tested. Long persistence of phytotoxic concentrations of this chemical in the soil may limit its widespread use as a greenhouse fumigant, but should not be a serious factor in many field applications. DD mixture and methyl bromide solutions also gave excellent control, although considerably larger dosages were required than with ethylene dibromide. Other fumigants such as tetrachlorethane and dichlorisopropyl ether were only moderately nematocidal and persisted in the soil for far too long a time to have practical value. Chloropicrin gave inconsistent and disappointing results, reasons for which are discussed later.

EFFECT OF THE DISINTEGRATION OF THE NEMATODE GALLS OF THE PREVIOUS CROP ON THE EFFICACY OF VARIOUS FUMIGANTS

Inconsistent nematode control was obtained with chloropicrin in the tests just described (Table 2). Since Godfrey and others[3] and Taylor[4] indicate that complete decay of the infested roots of the previous crop is necessary for best results with chloropicrin, several experiments were conducted to establish more clearly the extent to which galls must decay for chloropicrin treatment to be most effective. In these experiments the ability of methyl bromide and DD mixture to penetrate intact galls was also studied. The procedure consisted of placing large nonrotted nematode galls in gallon crocks of soil, treating different crocks at various time intervals, and then testing the efficacy of the treatment by setting indicator plants in the treated soil and counting the number of nematode galls that subsequently developed on their roots.

For the first experiment 68 one-gallon crocks were filled with moist, sandy loam soil, and ten large nematode galls obtained from recently dug tomato roots were distributed in the soil in each crock. The crocks were stacked and covered to minimize water loss from the soil. On the day the galls were placed in the soil four crocks were treated with chloropicrin by placing 0.7 ml. of the liquid in a hole 3½ inches deep in the center of each crock. Four

[3] Godfrey, G. H., J. Olivera, and H. Hoskins. Increased efficiency of chloropicrin for nematode control with better confinement of the gas. Phytopath. 24: 1332–1346. 1934.

[4] Taylor, A. L. Soil fumigation with chloropicrin for control of the root-knot nematode, Heterodera marioni. Phytopath. 33: 1166–1175. 1943.

crocks were treated in the same way using 3 ml. of a 10 per cent methyl bromide solution per crock. Following treatment the hole was closed with soil and the surface "sealed" with 100 ml. water. At the same time four nontreated crocks of soil were set aside as checks. At four-day intervals thereafter other crocks were treated in the same manner, except that 1 ml. chloropicrin was used per crock. The soil temperatures were 60° to 70° F. when treatments were made. Four crocks were left untreated at each of the 12- and 24-day intervals. Each time a treatment was made sample galls were removed from similar crocks of soil and examined to determine the extent of disintegration. On the day the galls were placed in the soil they were very firm. After four days a few galls were soft, but most of them were still fairly solid. In 8 days all of the galls were soft or mushy, and by 12 days they were easily broken apart and were becoming dry. After 16 days the galls were practically dry, consisting only of a central core and

TABLE 3.—*Effect of degree of decay of nematode galls upon the efficacy of chloropicrin and methyl bromide soil treatment*

Rotting period, in days before treatment	Average number of nematode galls on plants from treated crocks	
	Chloropicrin	Methyl bromide
0	223.0	0.5
4	100.5	0.0
8	13.0	0.0
12	177.3	0.8
16	284.3	3.3
20	12.3	2.0
24	0.0	0.3

thin layer of epidermal tissue, the subepidermal tissue being completely disintegrated. In 20 days the galls were thoroughly disintegrated, with only the hard central core of the root intact.

About two weeks after each treatment a tomato seedling was set in each crock. Approximately 50 days later the plant was carefully removed from the soil and the number of nematode galls counted (Table 3).

Poor nematode control was obtained by chloropicrin treatments made on the day the galls were placed in the soil, and also after the galls had stood in the soil for only 4 days. Fairly good control was obtained by the treatment on the 8th day, but treatments on the 12th and 16th days were again ineffective. It is interesting that these poor results were obtained at these later intervals, because by the 12th day the galls were fairly well disintegrated. One possible explanation is that the nematode has a stage in its cycle which is resistant to chloropicrin and which was most prevalent 12 to 16 days after the galls were placed in the soil. The poor nematode control by the first two treatments could be attributed to poor penetration of the chloropicrin into the intact galls. Good nematode control was obtained by all the methyl bromide treatments, indicating the ability of this fumigant to

penetrate intact galls, and the high toxicity of this chemical to all stages of the root-knot nematode. The plants set in the nontreated soil were in each case so severely injured by nematodes that the number of galls could not be counted.

In order to determine whether the poor results with chloropicrin were due to the inability of the vapors to penetrate intact galls or to a resistant stage in the life cycle of the nematode, and to check on the data obtained with the methyl bromide solution, a second experiment was conducted. The soil in each of 72 crocks was infested with 10 large nonrotted nematode galls as in the previous trial. In addition, a second series of 40 crocks was infested, each with the macerated pieces of nematode galls obtained by grinding 10 large galls through a food chopper. Crocks containing whole galls and macerated galls were treated at 4-day intervals, using 1 ml. chloropicrin

TABLE 4.—*Effect of degree of decay of nematode galls and of macerating galls upon the efficacy of chloropicrin and methyl bromide soil treatments*

Rotting period, in days before treatment	Average number of nematode galls on plants from treated crocks				
	Chloropicrin		Methyl bromide	Untreated	
	Whole	Chopped[a]	Whole	Whole	Chopped
1	0.3	0.0	0.0	358.0	835.0
5	100.0	3.3	10.3	.....	.....
9	543.0	1.0	2.0	.....	.....
13	197.3	29.3	0.8	.....	.....
17	70.5	0.3	0.0	.....	.....
21	9.0	0.0	0.0	.....	.....
25	0.0	0.0	2.0	.....	.....
30	0.0	0.0	56.0	386.6	524.5

[a] Nematode galls ground with a food chopper before being placed in soil.

per crock, as previously described. Also, crocks containing whole galls were treated at 4-day intervals, using only 1.5 ml. of the 10 per cent methyl bromide solution. The soil temperature during storage period varied between 70° and 75° F. Three small tomato seedlings were set in each crock two weeks after treatments. After they had grown for about 5 weeks the number of galls on their roots was counted (Table 4).

Again, the efficacy of chloropicrin treatment cannot be entirely correlated with the disintegration of the galls, since excellent control was obtained by the first treatment made in crocks containing whole nonrotted galls. The galls at that time contained mostly young eggs, and only a few live larvae. Poor control was obtained by treatments made on the 5th, 9th, and 13th days. By the 9th day the galls had disintegrated and many young larvae and mature eggs were present. It appears that inability of the chloropicrin to penetrate galls is an important factor, because satisfactory control, except on the 13th day, was obtained in the crocks containing the ground inoculum. The good control on the first day in crocks containing solid galls can be explained by assuming that the nematodes at the time were in a very suscep-

tible stage and, consequently, a very low concentration of chloropicrin was sufficient to be lethal. Later when the nematodes developed to a more resistant stage, the vapor that penetrated was insufficient. These experiments would indicate that a resistant stage of the nematode, as well as poor penetration of roots may be responsible for poor results when chloropicrin treatment is made too soon after the previous crop has been removed.

The ability of methyl bromide to successfully penetrate nonrotted nematode galls was again demonstrated. No explanation can be made for the poor control obtained on the 30th day. The ability of methyl bromide to penetrate nonrotted galls is of considerable economic value. This fact enables treatment to be made as the previous crop is removed. Furthermore, the rapid dissipation of methyl bromide from the soil permits replanting shortly after treatment.

TABLE 5.—*Effect of degree of decay of nematode galls upon the efficacy of three soil treatments*

Rotting period, in days before treatment	Average number of nematode galls on plants in soil treated with			
	Chloropicrin	DD mixture	Dowfume G	Not treated
1	0.0	1.0	0.0	685
3	0.0			
5	0.25	0.0	0.25	
7	10.0			
9	2.5	0.0	0.0	
11	1.25			
13	3.5	0.5	0.5	
15	0.5			
17	0.25	1.5	8.75	
19	0.0			
21	0.0	1.25	0.75	
23	0.0			627

In a third similar experiment, the soil in each of 104 crocks was infested with 10 large firm nematode galls from tomato roots. At two-day intervals, four crocks were each treated with 1 ml. chloropicrin. At four-day intervals four more crocks were treated with 1 ml. of a 10 per cent methyl bromide solution, and four others with 0.5 ml. crude DD mixture. Each time a treatment was made sample galls were examined to determine which nematode stages were present. After 12 treatments had been made 3 squash seeds were planted in each crock. The squash plants were removed 15 days later and the nematode galls counted (Table 5).

Good nematode control was obtained by all treatments, probably because the temperature of the soil was higher than in previous experiments. Again, chloropicrin treatments made after the galls had rotted somewhat were less successful than those made soon after the galls were placed in the soil. All stages of the nematode were present when the galls were placed in the soil, but young eggs were most abundant. By the 5th day young larvae were plentiful. Most of the eggs had hatched by the 11th day. It appears, therefore, that the poorest control with chloropicrin was obtained at about

the time when the majority of the nematode eggs were reaching maturity and when the first instar larvae were well developed but had not yet broken out of the eggs. Like methyl bromide, crude DD mixture appears to be effective regardless of the condition of the galls or nematodes.

A laboratory experiment was conducted to determine if the free-living larvae are more susceptible to chloropicrin than larvae still within the egg shell. Two milliliters of a water suspension containing all stages of the root-knot nematode were placed in Petri dishes and 10 ml. of a chloropicrin solution, containing 1 gm. chloropicrin in 1000 gm. water, were added. The larvae in suspension were then watched under the microscope. All of the larvae ceased moving in less than ten minutes and were presumed to be dead. Young larvae and old larvae appeared to succumb equally rapidly. Ten minutes after all movement ceased the suspension was diluted with 100 ml. of water. All stages of the nematode were then removed from the solution by centrifuging and decanting and were transferred to Petri plates of 2 per cent water agar containing mercuric chloride (1 to 10,000) to prevent the growth of contaminants. These plates were examined 24 hours later and many live free-living larvae were present. They had undoubtedly developed from larvae that had been within their egg shells when exposed to chloropicrin. It appears, consequently, that the larvae within the egg shell are resistant to chloropicrin. No concrete data have been obtained regarding the relative susceptibility of young immature nematode eggs, but since excellent nematode control was obtained in several of the experiments when young eggs were most prevalent, it would appear that immature eggs are easily killed. In similar experiments with methyl bromide solution and DD mixture no live larvae were found on the day following treatment, which would indicate that all stages of the root-knot nematode are readily killed by these chemicals.

It may be concluded from these experiments that in the case of methyl bromide or DD mixture treatments it is not necessary to delay soil treatment until galls from the previous crop have rotted. Whereas, in the case of chloropicrin treatment it is desirable for consistent results to delay treatment for 10 to 20 days, depending upon soil temperature and moisture conditions, to allow for decay of the root galls and to permit the nematodes to develop to a susceptible stage.

TIME REQUIRED FOR METHYL BROMIDE TO PENETRATE NONROTTED NEMATODE GALLS IN THE SOIL

An indication of the rapidity with which methyl bromide penetrates nonrotted nematode galls in the soil was obtained by the following experiment. Twenty-eight one-gallon crocks of soil were each infested with ten large firm nematode galls from tomato roots. Twenty-four of the crocks were treated by placing 1.5 ml. of a 10 per cent methyl bromide solution in a hole 3½ inches deep in the center of each crock. The hole was closed with soil and the surface wet with 100 ml. water following treatment. Two hours after

treatment the galls were removed from four of the crocks treated with methyl bromide and transferred to four crocks of steamed soil. Galls were transferred from other groups of four crocks to steamed soil after 4, 8, 12, 24, and 36 hours, respectively. In four crocks the galls were left in the methyl bromide–treated soil. One week after treatments 3 small tomato seedlings were set in all crocks containing the nematode galls. After 5 weeks these tomato plants were carefully removed from the soil and the number of nematode galls on their roots counted (Table 6).

It appears that under the conditions of this experiment lethal concentrations of methyl bromide diffused through the soil and penetrated nonrotted nematode galls in four hours, and that a concentration sufficient to kill a high percentage of the nematodes had penetrated in two hours. Data such as these indicate the rapidity with which soil fumigants diffuse in the soil

TABLE 6.—*Time required for methyl bromide to penetrate nonrotted nematode galls in the soil*

Time nematode galls were held in soil treated with methyl bromide[a] (hours)	Average height of tomato plants grown in soil infested with nematode galls from treated soil (inches)	Average number of galls per crock on tomato plants grown in soil infested with nematode galls from treated soil
2	15.1	16.5
4	14.0	2.3
8	14.4	1.8
12	13.8	3.5
24	13.3	1.5
36	12.9	3.0
Galls left in treated soil	12.7	0.3
Untreated	8.1	207.5

[a] Dowfume G (10 per cent methyl bromide) used for this experiment.

and emphasize the importance of having optimum soil conditions at the time the fumigants are added to the soil, rather than at some time following treatment.

CONTROL OF ORGANISMS AT VARIOUS LEVELS IN THE SOIL

In addition to establishing the merits and limitations of various fumigants, greenhouse tests can be of value in obtaining fundamental information regarding the edaphic factors affecting the action of fumigants, the effect of water seal or covers, the diffusion of fumigants, etc. Two experiments dealing with the latter subject have been conducted.

In one experiment 36 one-gallon crocks of soil were each infested with 25 grams of macerated roots infested with root-knot nematode. In one-third of the crocks the inoculum was placed in a layer ½ inch below the surface of the soil, in a second group, it was placed 3½ inches deep, and in the third 6½ inches deep, *i.e.*, ½ inch from the bottom of the crock. Four of each group were treated by injecting 1 ml. chloropicrin 3½ inches deep in the center of each crock. Four of each group were treated with 1.5 ml. of a 10 per cent methyl bromide solution. The remaining crocks were left untreated. The

soil was lightly watered immediately following treatment. Ten days later 3 tomato seedlings were set in each crock. After growing for 5 weeks, they were carefully removed and the number of nematode galls on their roots counted. The data obtained (Table 7) indicate that poorest control was obtained in those crocks in which the nematodes were placed only ½ inch below the surface of the soil. The chloropicrin treatment was relatively less effective at the surface than the methyl bromide.

The relatively large number of galls that formed on the roots of plants set in nontreated soil in which the inoculum had been placed 6½ inches deep is of interest. The nematodes in these cases must have migrated up from the bottom of the crock, since practically all of the galls were on the older roots near the surface. This supports the belief that nematodes are attracted to plant roots, and it appears that the stimulus involved is active over a distance of at least several inches.

TABLE 7.—*Effect of depth of nematodes in the soil on the efficacy of chloropicrin and methyl bromide soil fumigation*

Treatment[a]	Depth of inoculum in soil (inches)	Average number of nematode galls per crock
None	0.5	294.8
	3.5	138.0
	6.5	115.0
Chloropicrin	0.5	37.3
	3.5	0.8
	6.5	0.0
Methyl bromide	0.5	3.3
	3.5	0.3
	6.5	0.5

[a] One ml. chloropicrin or 1.5 ml. methyl bromide solution injected 3.5 inches deep.

For another experiment a quantity of macerated nematode-infested roots were thoroughly mixed into a sandy soil. The soil was then placed in 3 cylindrical containers 17.5 inches in diameter and 28 inches deep. Also, 5 large intact nematode galls were distributed near the edge of the container at each 4-inch level in the soil. The soil in one container was treated with 5 ml. chloropicrin, another with 5 ml. crude DD mixture, and a third with 12.5 ml. of a 10 per cent methyl bromide mixture by placing the fumigants in a hole 4½ inches deep in the center of each container. The surface was lightly watered after treatment. Twelve days later the soil was removed in 2-inch layers and placed in gallon crocks. In each 2-inch layer there was sufficient soil to fill 2 crocks. The whole galls were removed from the treated soil and each placed in a 4-inch clay pot of steamed soil. Indicator plants were then grown in all lots of soil, and the nematode galls that developed on their roots were counted.

It appears (Table 8) that the maximum concentration of the fumigant develops at about the level at which the liquids are injected. Chloropicrin was generally ineffective probably because of the low dosage employed, but

it was least effective at the surface layer. Methyl bromide, on the other hand, was about as effective at the surface layer as at the application level but was poor at the lower levels. This would indicate the advisability of applying fumigants with very high vapor pressures, as methyl bromide, deeper in the soil than chloropicrin. DD mixture was effective at all levels at the dosage employed.

PERSISTENCE OF VARIOUS FUMIGANTS IN THE SOIL

Since soil fumigants employed to date are very toxic to higher plants as well as to the undesirable organisms, it is necessary to delay planting treated soil until the concentration of the fumigant has reached a noninjurious level.

TABLE 8.—*Level in the soil of maximum concentration of fumigants as indicated by their effect on the root-knot nematode*

Depth from which soil and galls were removed (inches)	Average number of galls on indicator plants					
	Chloropicrin treatment		DD Mixture treatment		Methyl bromide treatment	
	Soil[a]	Galls[b]	Soil	Galls	Soil	Galls
0–2	90.5	31.1	0.5	0.4	4.5	0.4
2–4	8.0		0.0		4.0	
4–6	8.0	11.6	0.0	0.2	4.0	1.0
6–8	53.5		0.0		3.5	
8–10	15.5	34.6	0.0	0.0	4.5	1.8
10–12	17.5		0.0		4.5	
12–14	38.0	39.2	0.0	0.0	10.0	20.6
14–16	26.0		0.0		16.5	
16–18	29.5	32.8	0.5	0.0	32.0	18.0
18–20	77.0		1.0		18.0	
20–22	64.5	43.6	0.0	0.4	22.0	38.7
22–24	44.5		5.0		15.5	
24–26	36.0		8.0		16.5	
26–28	64.0	25.2	15.0	1.2	36.0	28.6

[a] Number of galls on plants grown in treated soil after whole galls were removed.
[b] Number of galls on plants grown in soil infested with whole galls taken from the treated soil.

The time required for this is of considerable importance in certain agricultural practices, particularly in greenhouse culture. Consequently, an experiment was conducted to determine the relative persistence of various fumigants in the soil.

A number of crocks were filled with moderately moist, sandy mineral soil or with muck soil. Three of these were treated with each of the dosages of the chemicals indicated in table 3 by placing the liquid in a hole 3½ inches deep in the center of each crock. Following treatment the hole was closed with soil and the surface wet with 100 ml. water. Three days prior to treatment a row of 5 wheat seeds was planted in each crock. Then just prior to treatment a second row was planted, and at 2-day intervals after treatment additional rows were planted in each crock. The soil was kept moderately moist throughout the experiment. About 3 weeks after treatment observa-

tions were made to determine the planting day that permitted normal growth of the wheat seedlings (Table 9).

Three factors appear to be of primary importance in determining the relative persistence of phytotoxic concentrations of various fumigants in the soil: (1) the vapor pressure of the fumigant, (2) the amount of fumigant

TABLE 9.—*Persistence of toxic concentrations of various fumigants in muck and sandy soils*

Treatment	Dosage[a] (ml.)	Boiling point (degrees C.)	Relative persistence in soil[b]
Muck soil treatment			
Check	.....	.....	−3
Dowfume G	0.75	43–85	−3
Xylene	1.50	138.8	0
DD Mixture	0.50	95–150	2
Chloropicrin	0.50	112.4	4
Sandy soil treatment			
Check	.....	.....	−3
Methyl bromide—propylene dichloride (1–9)	0.75	43–97	0
	1.50		0
Methyl bromide-xylol (1–9)	0.75	43–138	0
	1.50		0
Methyl bromide-alcohol (1–9)	0.75	43–78	−3
	1.50		2
Dowfume G	0.75	43–85	0
	1.50		2
Toluene	2.50	110.8	0
	5.00		2
Xylene	1.50	138.8	2
	3.00		2
DD Mixture (crude)	0.50	95–150	2
	1.00		2
Chloropicrin	0.50	112.4	2
	1.00		4
Tetrachlorethane	0.75	146.3	6
	1.50		8
Ethylene dibromide	0.25[c]	131.7	14+
	0.50		14+
Dichlorisopropyl ether	0.75	187.1	14+
	1.50		14+

[a] Amount of chemical injected per crock.

[b] Figure indicates number of days after soil treatment when wheat seed could be planted without injury. Negative values indicate days prior to treatment.

[c] Subsequent work has shown this to be an excessive dosage and consequently the period of persistence would be considerably shorter.

applied, and (3) the concentration that plants are able to tolerate. The vapor pressures of fumigants are, in general, negatively correlated with their boiling points, and the boiling point can, therefore, be used as a good criterion for judging volatility and the rapidity with which fumigants will escape from the soil. In general, fumigants that boil at about 100° to 120° C. will dissipate in a reasonable period of time, and those boiling at 130° to 140° C. or over will persist too long. However, the lower the total

dosage of the fumigant employed, and the smaller the amount applied per injection, the sooner a subtoxic concentration to plants is reached. With fumigants extremely toxic to undesirable organisms, as ethylene dibromide to nematodes, the feasibility of using very low dosages compensates for low vapor pressure to some extent. Weak toxicants, as xylene, while tolerated in higher concentrations by plants, must be employed in such high dosages for good nematode control that long aeration periods may be required. Fumigants with low boiling points and low plant toxicities, as methyl bromide, have a distinct advantage in many practices in permitting earlier planting. The ideal fumigant to control undesirable organisms in the presence of annual plants has yet to be found.

Soil conditions have a pronounced effect on the length of the aeration period. Aeration is slower in cold soils because of the reduced vapor pressure of the fumigant, and in soils having low free porosity, such as wet, heavy soils, because of poor diffusion of the fumigant. Some indication of the marked effect of soil conditions may be obtained by citing field experience with chloropicrin where in warm sandy soils, planting has been done 3 days after treatment without injury, whereas under adverse conditions injury has occurred when planting was delayed a month or more. The order in which a group of fumigants may be expected to dissipate from the soil will not be changed by soil conditions, but the relative rates may change, and in general the higher the boiling point of the fumigant, the greater the effect of adverse conditions.

DISCUSSION

It is recognized that there are certain limitations to the type of greenhouse tests herein described. However, it is believed that by means of such tests an accurate evaluation of the relative efficacies of fumigants against various organisms can be obtained. Field trials are desirable to determine the optimum dosage of a new fumigant under commercial application, but if the greenhouse tests indicate, for example, that 2 ml. of a new fumigant must be employed to achieve the same results obtainable with 1 cc. of chloropicrin, about the same relative dosages will be required under field conditions. Some variation in effectiveness may be expected, however, depending on such edaphic conditions as texture, moisture, and temperature, and in general the lower the vapor pressure of the fumigant the more it is affected by adverse soil conditions.

It may be argued that in small containers the ability of a fumigant to diffuse in the soil is not satisfactorily tested. However, since the dosage employed is decreased in proportion to the required diffusion distance, if a chemical still develops a lethal concentration throughout the container, it is safe to assume that it will diffuse the required distance in the field. That these tests will indicate the inability of a fumigant to diffuse is evident from the failure of formaldehyde when injected to control damping-off, which must be attributed to poor diffusion since good control was obtained when

this compound was mixed throughout the soil. Of course it is not possible to determine the optimum spacings or depths of application for fumigants without resorting to treating larger soil masses.

It is difficult to obtain a satisfactory evaluation of relatively nonvolatile solid materials by these tests because the thoroughness with which such chemicals are incorporated with the soil is an important factor in determining their effectiveness. The small greenhouse tests, where thorough mixing is possible, tend to overestimate the value of a compound, since in the field it is often impractical to obtain the required degree of mixing. Some standard mixing procedure should probably be employed to avoid incorporating the powder more thoroughly than can be accomplished in the field.

SUMMARY

A number of soil fumigants were compared in greenhouse experiments designed to test the merits and limitations of soil fumigants.

Chloropicrin was the most effective fungicide of the fumigants tested. DD mixture and methyl bromide solutions were also fungicidal, but only at considerably higher dosages than are required for nematode control.

Ethylene dibromide was the most efficacious nematocide, followed by DD mixture, methyl bromide solutions, and chloropicrin in descending order of effectiveness.

For consistent effective root-knot nematode control with chloropicrin it was necessary to delay treatment for 10 to 20 days following the removal of the previous crop to allow for decay of the root galls and to permit the nematodes to develop to a susceptible stage. Methyl bromide solutions and DD mixture were effective regardless of the condition of the galls. A lethal concentration of methyl bromide diffused through the soil and penetrated large intact nematode galls in 2 to 4 hours.

Maximum concentrations of fumigants developed in the soil at about the level to which they were injected. With chloropicrin treatment the surface of the soil was less effectively fumigated than were the lower levels. Methyl bromide, while about as effective near the surface as at the injection level, gave poorer results at the lower levels.

The persistence of phytotoxic concentrations of the fumigants in the soil was found to depend on the vapor pressure of the fumigant, the amount applied, and the concentration that plants are able to tolerate.

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THE CONTROL OF CABBAGE DOWNY MILDEW THROUGH THE USE OF SPRAYS[1]

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INTRODUCTION

Cabbage downy mildew (*Peronospora parasitica* (Fr.) Tul.), better known to Mississippi truck growers as "rust," occurs each year in the Copiah County trucking area, frequently causing growers severe loss. Truck farmers have considered downy mildew as the most serious cabbage disease in Mississippi. If mildew first appears during late December and weather conditions are unfavorable for rapid disease development, cabbage plants may not be killed since mildew develops slowly on older plants, but transplanting from coldframes to the field may be delayed.

Downy mildew has been an important factor in causing approximately one-half of the Copiah County cabbage acreage to be set with cabbage plants grown outside this area. The estimated ten-year average for cabbage in Mississippi is 6,940 acres; the greatest acreage to date, 8,300, was set in 1944. An efficient and economical control measure would insure individual growers of healthy, mildew-free cabbage plants.

LITERATURE REVIEW

Downy mildew has previously been reported (7) as severe in Mississippi. The disease, under southern conditions, has previously been described (2, 3) and environmental factors affecting downy mildew of cabbage have been determined (4). Eddins (2, 3) has shown that spraying is both successful and practical in Florida. In his earlier publication (2) he recommended Spergon (tetrachloro-parabenzoquinone) and Fermate (ferric dimethyl dithiocarbamate) applied as spray or dust but stated that "the sprays have proved more effective than the dusts." In a later paper (3) he limited his recommendation to Spergon applied either as a spray or dust.

Although Anderson (1) showed Fermate to be an effective fungicide for the control of downy mildew of tobacco, this organic fungicide has not been equally effective in the control of cabbage downy mildew. In his first recommendation Eddins (2) stated that "Spergon treated plants have grown more rapidly and have been freer of mildew than those sprayed or dusted with Fermate." Later he reported (3) Spergon as the only fungicide tested which was found to give satisfactory control.

Previously, mention has been made by Foster and Pinckard (5) of the control of cabbage mildew by means of benzene vapor and a separate report regarding the action of benzene vapor has been prepared (6). The present paper is limited to the control of cabbage mildew by means of sprays.

[1] Contribution from the Truck Crops Branch Experiment Station, Crystal Springs, Mississippi. Published with the approval of the Director, Mississippi Agricultural Experiment Station, State College, Mississippi. Paper No. 129, New Series.

METHODS AND MATERIALS

During the past two years eight different fungicides (Table 1) have been tested in the greenhouse for the control of cabbage downy mildew. Spergon (wettable) and Dow Seed Protectant No. 5 contain the same active ingredient (tetrachloro-parabenzoquinone) though in different proportions and with different amounts of emulsifier. The spreaders Orvus and Vatsol O.T.C. were used at the rate of ½ pound per 100 gallons. Following repeated greenhouse tests the more effective fungicides were further tested under outside coldframe or seedbed conditions.

TABLE 1.—*The efficacy of fungicidal sprays, applied three times a week, in controlling downy mildew of cabbage in the greenhouse*

Fungicide[a]	Rate per 100 gal.	No. of trials	Spreader	Disease rating[b]
	Lb.			
Yellow Cuprocide	1	5	Vatsol	2
Dithane B-11[c]	1	5	Orvus	0–1
Spergon (wettable)[d]	4	5	Vatsol	0–1
Dow Seed Protectant No. 5[d]	2	5	Orvus	0–1
Dow Seed Protectant No. 6[e]	½	3	Orvus	1–2
Fermate[f]	2	3	Vatsol	2–3
Phygon (wettable)[g]	¼	2	Vatsol	3
AO3 (wettable)[h]	1	2	Vatsol	2–3
Unsprayed control	.....	8	.....	3–4

[a] Fungicides donated without cost through the courtesy of the following: Rohm & Haas Company; United States Rubber Company, Naugatuck Chemical Division; E. I. du Pont de Nemours & Co., Grasselli Chemicals Department; and The Dow Chemical Company.

[b] Key for disease rating:
0 = No fungus sporulation, no apparent spray injury.
1 = Trace to slight sporulation and/or trace to slight spray injury.
2 = Slight to moderate sporulation and/or slight to moderate spray injury.
3 = Moderate sporulation, few plants killed, and/or moderate to severe spray injury.
4 = Abundant and general sporulation, few to many plants killed, and/or very severe spray injury.

[c] Experimental material containing Disodium ethylene bis-dithiocarbamate, zinc sulfate monohydrate, and hydrated lime.

[d] Tetrachloro-parabenzoquinone.

[e] Trichlorophenol.

[f] Ferric dimethyldithiocarbamate.

[g] 2,3-dichlor-1,4-naphthoquinone.

[h] Phenanthraquinone.

In both greenhouse and seedbed experiments cabbage varieties grown commercially in this area were used, such as Golden Acre, Copenhagen Market, and Round Dutch. For greenhouse experiments cabbage seed were sown in 4-inch clay pots. Usually four to eight pots of cabbage seedlings (approximately 30 seedlings per pot) were used in testing each fungicide. An unsprayed control was used for a comparative check. Spray applications were begun shortly after the cotyledons appeared, and in advance of mildew sporulation. In greenhouse trials all plants were inoculated with a spore suspension following the third spray application. Plants were kept

shaded and moistened for approximately 24 hours following inoculation. In the greenhouse the fungus usually sporulated abundantly on the cotyledons of unsprayed (control) plants within five days following inoculation. Spray treatments were continued, usually 6 to 12 applications, until unsprayed control plants were killed or surviving control plants began to recover from the mildew disease. In the seedbed, sprays were applied until plants were of size for field transplanting, usually until they had four or five true leaves.

In early greenhouse experiments a weekly schedule of three spray applications was maintained. Later greenhouse experiments indicated that two spray applications a week with Dithane B-11, Spergon (wettable), and Dow Seed Protectant No. 5 would give practical mildew control.

Early in the spring of 1945 an experiment was designed to compare four different fungicides in an outside seedbed, applied at two different frequencies, each fungicide being applied in duplicate on one-square-yard units. Golden Acre variety was sown February 10, and final data were taken May 2 and 3, 1945. Mildew developed following combined artificial inoculation and natural infection. In the fall of 1945 three spray experiments were conducted on cabbage seedbeds sown at three different dates.

In seedbed experiments all comparable units were sown with a uniform weighed amount of seed. Data were obtained by recording number of plants for one square foot, weight of plants cut at soil line per one square foot, and weight of 100 large plants selected at random and cut at the soil line. A disease rating based on a scale of zero to four was used for both greenhouse and plantbed experiments.

PRESENTATION OF DATA

A. *Greenhouse Trials.* In repeated greenhouse trials three fungicides, Dithane B-11, Spergon (wettable), and Dow Seed Protectant No. 5 (tetrachloro-parabenzoquinone), resulted in a very low disease rating, indicating a high degree of control. Based on Florida reports (2, 3) Spergon (wettable) was expected to give good control at three applications a week. Dow Seed Protectant No. 5, at the rate of 2 lb. to 100 gal., gave about equally good control as Spergon (wettable) at 4 lb. per 100 gal. Among the fungicides tested in the greenhouse, Dithane B-11 at 1 lb. to 100 gal. gave perhaps the best control. However, as shown in table 2, Dithane B-11 failed to give satisfactory control in outside seedbeds. Other fungicides tested gave more or less control as compared to unsprayed plants but were definitely inferior to Spergon (wettable) and Dow Seed Protectant No. 5. Dow Seed Protectant No. 6 failed to prevent sporulation, suggesting that under more severe conditions control would be inadequate. Fermate was definitely inferior in control. Two additional fungicides from U. S. Rubber Company, Phygon (wettable) and No. AO3 (wettable), induced some injury and failed to prevent sporulation. Plants in unsprayed control pots were 100 per cent infected, which resulted in abundant sporulation. Usually many control plants were killed. Results of greenhouse sprays, three applications each week, including explanation of disease rating, are summarized in table 1.

During the course of these investigations several emulsifiers and spreaders were used, certain of which were more satisfactory than others. With certain fungicides the emulsifier or spreader was of major importance. For example, Dow Seed Protectant No. 5 failed to make a satisfactory suspension when mixed directly with water or with water and certain emulsifiers such as B-1956. However with Orvus used as an emulsifier Dow No. 5 produced a finely divided suspension and proved to be an effective fungicide in the control of downy mildew. With several fungicides, including Spergon (wettable), Vatsol O.T.C. gave excellent results as an emulsifier and spreader. In preliminary tests self-emulsifying cotton-seed oil (one per cent solution) appeared markedly toxic to cabbage seedlings, inducing stunting and malformation of young cabbage plants.

B. *Seedbed Trials.* In the first seedbed experiment it was attempted to determine if two spray applications a week would give approximately as good control as sprays applied three times each week. A total of six and eight spray applications were applied to one-square-yard units receiving two and three spray applications a week, respectively. Four fungicides were used, the three fungicides giving the best control in previous greenhouse studies and Fermate. All treatments were in duplicate. In this experiment both Spergon (wettable) and Dow No. 5 induced satisfactory control. With Spergon (wettable) both stand and weight of plants were greatest when sprays were applied three times a week. However, the disease rating was the same for two spray applications a week, suggesting that irregular spacing of seed might, in part, explain the difference in results. Dow No. 5 plots had approximately the same average stand count whether they had two or three spray applications a week. Weight of plants per square foot and weight of 100 plants pulled at random was in favor of two spray applications each week. The average disease rating was the same in all treatments for Dow No. 5 and Spergon (wettable), giving additional confirmation to the high degree of control these compounds induced under greenhouse conditions. Fermate resulted in relatively poor control when compared with Dow No. 5 and Spergon (wettable), except for the number of plants per square foot following three spray applications a week. In that treatment the plant stand was rather high but plants were so small and stunted that plant weight was very low. The disease rating for both Fermate treatments was 3. Results from the unsprayed control plots show that within the two control units the total number of surviving plants per square foot were 6 and 34, respectively. The control units had a disease rating of 4. This is good evidence of an exceedingly severe mildew attack. Results of this experiment, including disease rating, are shown in table 2. This experiment strongly suggests that under severe conditions two spray applications each week of the more effective fungicides, when carefully applied, will satisfactorily control cabbage downy mildew.

Additional evidence relating to the effective control of cabbage downy mildew by spraying was obtained during the fall and winter season of

1945–46. In the first spray experiment a seedbed of approximately six square yards was sown to Round Dutch on October 15, and divided into three units; the two outer sections were sprayed with Spergon (wettable) and Orvus (emulsifier); the central section remained the unsprayed control. Cabbage mildew developed following natural infection. A total of six spray

TABLE 2.—*The efficacy of fungicidal sprays in controlling downy mildew of cabbage in the plant bed, in 1945*

Treatment	Av. no. of plants per 1 sq. ft.	Av. wt. of plants per 1 sq. ft. (above soil line)	Av. wt. of 100 plants pulled at random (above soil line)	Average disease rating[c]
		Grams	*Grams*	
Dithane B-11,[a] 1 lb. to 100 gal., twice a week	127	34	31	3
Dithane B-11,[a] 1 lb. to 100 gal., 3 times a week	137	47	41	3
Spergon (wettable), 4 lb. to 100 gal., twice a week	243	115	67	0–1
Spergon (wettable), 4 lb. to 100 gal., 3 times a week	274	152	86	0–1
Dow Seed Protectant No. 5, 2 lb. to 100 gal., twice a week	226	139	87	0–1
Dow Seed Protectant No. 5, 2 lb. to 100 gal., 3 times a week	227	132	78	0–1
Fermate, 2 lb. to 100 gal., twice a week	155	55	42	3
Fermate, 2 lb. to 100 gal., 3 times a week	216[b]	61	39	3
Unsprayed control	20	7	7	4

[a] Dithane B-11 was strictly experimental material and should not be confused with the more recent product, Dithane D-14.

[b] Very small weak plants.

[c] Key for disease rating:

0 = Vigorous plant growth, no sign of infection.

1 = Vigorous plant growth, 1 or 2 lower true leaves occasionally with trace or slight necrosis. All true leaves holding well, stems bright. No fresh fungus sporulation.

2 = Rather vigorous growth, occasional lower true leaf with necrotic lesions and with some chlorosis, occasional lower leaf ready to drop or dropped. Little or no killing of plants in early seedling stage, stems bright. Usually no fresh sporulation.

3 = Growth fair to poor, more or less stunting, usually a few plants killed during early seedling stage. Necrosis mostly general on true leaves, with one or more lower leaves usually dropped. Stem discoloration frequent. Fresh sporulation may or may not occur.

4 = Marked stunting, usually many plants killed in early seedling stage. Most plants with abundant necrosis on true leaves, lower leaves usually dropped. Plants frequently weak and stems discolored. Secondary rots may be present on stems and roots. Fresh sporulation may or may not occur.

applications were applied twice a week; the first spray was applied November 2 and the final spray on November 19, 1945. Relatively high temperatures forced growth of cabbage plants to field transplanting size by November 20, 1945, when final data were taken.

Data based on a single sample taken from a unit sprayed with Spergon

(wettable) showed 280 plants per square foot, with plant tops weighing 209 grams, and tops from 100 large plants pulled at random weighed 112 grams. The disease rating was 1 to 1.5. In comparison, the unsprayed unit gave 199 plants per square foot, with plant tops weighing 78 grams, and 100 large plants pulled at random weighed 52 grams. The disease rating was 3. These results indicate that Spergon (wettable) is a markedly effective fungicide in the control of cabbage downy mildew.

The second spray experiment of the 1945–46 season was also with Round Dutch cabbage, sown on October 31, 1945. Treatments, including control, were in duplicate on units of one square yard. Cabbage beds were inoculated on November 11, and the first spray was applied November 12, 1945. Eleven sprays were applied; the final application was made on December 31, 1945. Sprays were applied twice a week but because of rain several applications were necessarily omitted. Final data were taken January 29, 1946. In this experiment Spergon (wettable) and Dow No. 5, with Orvus as an emulsifier, were compared with an unsprayed control. The unsprayed con-

TABLE 3.—*Comparison of Spergon (wettable), Dow No. 5, and unsprayed control. Data taken January 29, 1946*

Treatment	No. of plants per 1 sq. ft.	Wt. of plants per 1 sq. ft. above soil line	Wt. of 100 plants above soil line (large plants)	Average disease rating[a]
		Grams	*Grams*	
Spergon (wettable), 2 sprays a week	120	343	625	1.5
Dow No. 5, 2 sprays a week	99	259	586	1.5
Unsprayed control	93	160	412	3

[a] Disease rating. See table 2, footnote c.

trol produced 93 plants per square foot, weighing 160 grams, as compared with 99 plants weighing 259 grams and 120 plants weighing 343 grams for Dow No. 5 and Spergon (wettable), respectively. Weight of 100 plants pulled at random was markedly less for the untreated control. In this experiment Spergon (wettable) seemed to induce better plant growth, based on plant weight, than Dow No. 5. Data are presented in table 3.

The final and third experiment during the 1945–46 season was with the Golden Acre variety. A large farm bed of 66 square yards was sown with one pound of seed November 20, 1945. Previously the soil in one-half of the bed had been treated with chloropicrin. Four different sprays, Yellow Cuprocide, Spergon (wettable), Dow No. 5, and Fermate, were compared with unsprayed controls. All treatments were in duplicate on units of approximately five square yards and all units were inoculated with a spore suspension. The first sprays were applied on December 7; the plants were artificially inoculated on December 12, 1945. A total of 12 sprays were applied; several sprays were omitted because of rain. Final sprays were applied February 5, and final data were taken 13 to 15 days later. All treat-

ments were tested on both chloropicrin-treated soil and nontreated soil. Orvus, as an emulsifier, was used with all sprays. Results of this experiment are presented in table 4.

Data from this trial show that Spergon (wettable) gave effective control on both chloropicrin-treated and nontreated soil. The average number and weight of plants per square foot was in favor of chloropicrin-treated units, while weight of 100 plants pulled at random was in favor of units without soil treatment. Dow No. 5 also gave number and weight of plants per square foot in favor of units without chloropicrin soil treatment. The weight

TABLE 4.—*Comparison of four different fungicides applied twice a week in the control of cabbage downy mildew. Data taken February 18–20, 1946*

Treatment	Av. no. of plants per 1 sq. ft.	Av. wt. of plants per 1 sq. ft. (above soil line)	Av. wt. of 100 plants pulled at random (above soil line)	Average disease rating[c]
		Grams	*Grams*	
Yellow Cuprocide,[a] 1 lb. to 100 gal.	118	109	164	3
Yellow Cuprocide,[b] 1 lb. to 100 gal.	112	213	341	2
Spergon (wettable),[a] 4 lb. to 100 gal.	164	256	345	1.5
Spergon (wettable),[b] 4 lb. to 100 gal.	244	282	293	1.5
Dow Seed Protectant No. 5,[a] 2 lb. to 100 gal.	187	265	285	1.5
Dow Seed Protectant No. 5,[b] 2 lb. to 100 gal.	137	258	296	1.5
Fermate,[a] 2 lb. to 100 gal.	142	128	154	3
Fermate,[b] 2 lb. to 100 gal.	109	117	174	3
Unsprayed control[a]	148	91	121	3–3.5
Unsprayed control[b]	121	106	178	3

[a] Soil was not treated with chloropicrin.
[b] Soil was treated with chloropicrin.
[c] Disease rating. See table 2, footnote c.

of 100 plants pulled at random was greatest for units receiving chloropicrin soil treatment. Fermate and Yellow Cuprocide gave relatively poor control. Data for unsprayed control units indicated that plants were markedly small and weak. In comparing data from units receiving chloropicrin soil treatment the unsprayed units gave an average of 121 plants per square foot, weighing 106 grams, while units sprayed with Spergon (wettable) gave an average of 244 plants per square foot, weighing 282 grams.

Both Spergon (wettable) and Dow Seed Protectant No. 5 induced a similar pattern on leaves of cabbage seedlings. The pattern was characterized by rather narrow, long leaves with a pronounced vein clearing effect. It was first observed following several spray applications but did not appear to adversely affect the growth of the cabbage plants. Plant growth following field transplanting appeared normal.

EQUIPMENT AND COST

Eddins (3) stated that best results were obtained from sprayers delivering at least 200 lb. pressure. He considered poor control likely to result if small hand sprayers were used. Spray sufficient to cover 100 square yards of small cabbage seedlings was estimated at 3 to 4 gallons. Later when plants were 4 to 8 inches tall he considered that 7 to 12 gallons were required to cover 100 square yards.

A power sprayer delivering spray at 200 lb. or greater pressure would be desirable. However, such equipment is not considered essential. In these trials all experimental spraying was with hand and cart sprayers delivering an estimated maximum of 50 lb. pressure. From the results of these experiments the writer is convinced that under Mississippi conditions satisfactory control can be obtained with low pressure equipment providing spray applications are started in advance of sporulation, sprays are applied regularly, and the spraying is done carefully and thoroughly.

In Mississippi, cabbage is seeded by growers in covered beds or coldframes of 60 to 70 square yards. For 70 sq. yd. of small cabbage seedlings it is estimated that 4 to 7 gallons of spray should be used. The amount of spray should be increased for each application since complete coverage is essential. For large cabbage plants, shortly before transplanting, an estimated 12 to 15 gallons of spray should be applied to each 70 square yards.

Basing the cost on present retail prices, Spergon (wettable) required to spray 70 sq. yd. of cabbage seedlings 12 to 15 times would cost the grower approximately four to five dollars or about 17 cents per thousand plants, and require a total of 100 gallons of spray. A bed of 70 square yards should set at least three acres, 10,000 plants to the acre. In recent years cabbage plants have frequently sold at two to four dollars per thousand. Spraying is therefore a cheap control measure.

DISCUSSION

Experimental data bring out certain facts in regard to fungicidal control of cabbage downy mildew. Fungicides tested varied greatly in their ability to induce effective control; also the compatibility of fungicide and emulsifier was found to be highly important.

Under Florida conditions Eddins (2) first recommended Spergon and Fermate as fungicides for the control of cabbage downy mildew. Later he (3) limited his recommendation to Spergon, recommending Spergon spray at the rate of 2 lb. to 50 gallons of water. Under very severe conditions he found that mildew was not satisfactorily controlled unless plants were sprayed three times a week while in the plant bed. Under Florida conditions it was reported safe to suspend treatments when night temperatures dropped below 40° F. Immediately following a rain, spraying was essential in order to control the disease.

In both greenhouse and plant bed trials the writer found Spergon (wettable) and Dow Seed Protectant No. 5 to give satisfactory control when

applied twice a week, as shown in tables 1 and 2. Although spray applications were started in advance of fungus sporulation, Fermate proved to be an unsatisfactory spray for the control of downy mildew.

The difference in humidity and possibly other environmental conditions may, in part, explain why Spergon (wettable) applied twice a week in advance of sporulation has given good control in Mississippi, while in Florida three spray applications a week are usually required.

SUMMARY

1. Eight different fungicides were tested in the greenhouse for the control of cabbage downy mildew.

2. Spergon (wettable) at 4 lb. to 100 gallons and Dow Seed Protectant No. 5 at 2 lb. per 100 gallons were found, under greenhouse and plant bed conditions, to give practical control when applied twice a week.

3. Several emulsifiers and spreaders were tested. Orvus was found to be compatible with Spergon (wettable) and Dow No. 5, and it was an effective spreader.

4. Data were recorded by taking number and weight of plants per square foot, weight of 100 large plants pulled at random, and disease rating based on a scale from zero to four.

5. An estimated amount and cost of spray required for a 70-square-yard bed is given.

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TOBACCO DOWNY MILDEW, ENDEMIC TO TEXAS AND MEXICO

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Endemism of the pathogen causing the downy-mildew disease of cultivated tobacco and the question as to the identity of this fungus have long been vexatious problems. In attempting to account for the outbreaks of downy mildew that occurred in the tobacco growing area of Georgia and Florida in 1921, and again in 1931, some have maintained that the causal fungus is in all probability indigenous to the United States. Other investigators have maintained, however, that the pathogen may have been introduced from Australia. It may be recalled that downy mildew disappeared after the first outbreak and did not recur until 1931. After the second outbreak it not only persisted but soon thereafter became widely dispersed throughout other tobacco growing regions. When Godfrey (7) discovered a downy mildew on a native tobacco, *Nicotiana repanda* Willd., in the lower Rio Grande Valley in 1941, it was immediately conjectured that this region in Texas probably constituted the reservoir of inoculum for these original outbreaks on cultivated tobacco in the Georgia-Florida area.

Pathologists interested in tobacco diseases have for several years considered the feasibility of eradicating downy mildew. The existence of a downy mildew on wild tobacco in the West is of primary significance in relation to success of efforts to eradicate the downy mildew of cultivated tobacco in the East. Unequivocal identification of the pathogen on wild tobacco therefore was necessary as a first step in formulating plans for eradication. Godfrey (7) was of the opinion that the downy mildew on *Nicotiana repanda* is *Peronospora tabacina* Adam, but positive identification was not possible since his collections consisted only of the sporangial stage. Because of these circumstances, the writer, in early March of the current year, made observations and collections of downy mildew on *N. repanda* in Texas, and has embodied the results of these studies in the present report.

HISTORICAL REVIEW

A downy mildew on cultivated tobacco is known with certainty to have existed in Australia since 1890, and presumably it occurred there 30 to 40 years previously (10). It is native there on *Nicotiana suaveolens* Lehm. This pathogen was early identified as *Peronospora hyoscyami* de Bary. De Bary (4) first employed this name for an European parasite, in the sporangial stage, on *Hyoscyamus niger* L., a solanaceous species related to *Nico-*

[1] Grateful acknowledgment is made of help given me by Dr. G. H. Godfrey and Mr. Walter J. Bach, Weslaco, Texas, also to Dr. B. C. Tharp and Dr. Fred A. Barkley for making available to me the collections of *Nicotiana* in the herbarium at the University of Texas, and to Dr. Harold N. Moldenke for compiling a list of specimens of *Nicotiana repanda* in the herbarium of The New York Botanical Garden.

tiana. Its oosporic stage was not found however, until some 50 years later when Bakhtin (3) described it from Russia.

In 1885, Farlow (6) observed on *Nicotiana glauca* Graham growing as an introduced species near San Diego, California, the sporangial stage of a downy mildew which he identified as *P. hyoscyami*. In the light of subsequent events it is now of unusual interest to recount that Farlow speculated on the possible consequences of the prevalence of this downy mildew by stating: "It is much to be feared that the disease (fungus) which attacks *Nicotiana glauca* may sooner or later extend to cultivated tobacco. If this happens the injury to tobacco will be very great. . . . If *N. glauca* and its parasite are once introduced into the Gulf States, the parasite might attack tobacco grown there and then pass on to Virginia and the other States where tobacco is the most important crop." A few years later Spegazzini (12) described, as occurring on *N. longiflora* Cav. growing near Buenos Aires, Argentina, an oosporic fungus that he named *Peronospora nicotianae*.

The outbreak, in 1921, of a downy mildew disease in tobacco seedbeds in the Georgia-Florida tobacco growing area was first reported by Smith and McKenney (11). They ascribed the disease to *Peronospora hyoscyami*. The collections of a downy mildew, identified as *P. hyoscyami*, on cultivated tobacco near Hallettsville, Texas, in 1906, and of one on *Nicotiana bigelovii* S. Watson in Nevada, in 1914 were mentioned in this report. The specimens gathered by Smith and McKenney during the 1921 outbreak lacked oospores, and failure of the fungus to produce oospores was employed by Clayton and Stevenson (5) as a likely explanation for the prompt disappearance of downy mildew from the Georgia-Florida area after this outbreak.

In Australia, Angell and Hill (2), as the result of inoculation experiments, found that more than a dozen species of *Nicotiana* are susceptible to attack by the downy mildew from cultivated tobacco, but that *Hyoscyamus niger* is immune. In their opinion these results cast grave doubt on identification of the tobacco downy mildew as *Peronospora hyoscyami*. The following year Adam (1) described the tobacco pathogen as a new species, *Peronospora tabacina*, on the basis of discovery that the organism possesses oospores of a kind that are strikingly different in size and in sculpturing of the walls from *P. nicotianae*, and of failure of the pathogen from tobacco to infect *H. niger*, as had been determined experimentally during the previous season by Angell and Hill (2).

The writer and his associates (16) in the first of their series of studies of tobacco downy mildew, also reported inability to secure infection on *Hyoscyamus niger* seedlings grown in a seedbed beside one in which tobacco seedlings became severely attacked by downy mildew. They concluded that the tobacco pathogen is not *Peronospora hyoscyami*, but instead is either *P. nicotianae* or some undescribed species. Spegazzini's type specimens of *P. nicotianae* were not available to us for comparison, and the report by Adam (1) in which he described *P. tabacina*, appeared while our report (16) was in press. The tobacco downy mildew, however, has been designated

P. tabacina in all subsequent reports of investigations in North America and in Australia. Moreover specimens of the sporangial stage of a downy mildew collected on *N. attenuata* Torr., in Nevada, in 1937, and others collected there in 1938, were identified as *P. tabacina* (13). Specimens on cultivated tobacco, containing oospores, were sent from Rio Grande do Sul, Brazil, in 1938 and were identified as *P. tabacina* by Wolf (15). The name *P. nicotianae*, however, was applied by Godoy and Coste (8) to the pathogen on cultivated tobacco grown in northern Argentina, in the State of Salta.

In 1943, Clayton and Stevenson (5) made critical comparative studies of 70 collections of tobacco downy mildew. Among 40 of these on cultivated tobacco, 9 were found to possess oospores. Among those collections from cultivated tobacco which did not possess oospores were specimens from the outbreak in Georgia-Florida in 1921, and one sent them from Rio Grande do Sul, Brazil. None of the remaining 30 collections involving 12 other species of *Nicotiana* was found to have oospores. These included specimens on *N. bigelovii* and *N. attenuata* from Nevada, as previously mentioned, and those on *N. repanda* sent from the Rio Grande Valley by Godfrey (7). Clayton and Stevenson (5) concluded (a) that neither sporangiophores, sporangia, nor oospores of *Peronospora tabacina* have distinctive morphologic features that could aid in specific determination, (b) that the evidence favors the view that this fungus is the only one on *Nicotiana* and (c) that *P. tabacina* is probably native to all temperate-zone regions having a native *Nicotiana* flora.

The observations of Clayton and Stevenson (5) on the paucity of oospores in collections of *Peronospora tabacina* accords with the findings of Angell and Hill (2) who noted only one collection with oospores among hundreds of collections of downy mildew on cultivated tobacco in Australia. The writer, however, does not regard the production of oospores as of rare occurrence, since he has never failed to find them in old decaying leaves in hundreds of specimens gathered from widely separated areas throughout the southeastern United States.

PRESENT OBSERVATIONS ON NATIVE TOBACCOS AND DOWNY MILDEW

The *Nicotiana* flora of the lower Rio Grande Valley, embracing the contiguous region in Mexico, includes five species. All of them are native or else were introduced and long ago escaped from cultivation. These species include *N. repanda* Willd., *N. glauca* Graham, *N. longiflora* Cav., *N. trigonophylla* Dunal., and *N. plumbaginifolia* Viv. Of these *N. repanda* seems to be the most abundant, has the widest range, and is represented in herbaria by the most numerous specimens, the first of which was collected nearly 100 years ago. The writer's collections of it, outside the Rio Grande Valley were made at Alice, Falfurrias, San Antonio, Austin, La Grange, and Hempstead. The range of this species in southern Texas can be closely approximated from figure 1. This map shows, by counties, the sites of all collections of *N. repanda*, represented by specimens in the herbaria of the University of Texas and the New York Botanical Garden.

Lesions of downy mildew on the leaves of *Nicotiana repanda* are scarce among the herbarium specimens examined, for the reasons that few collections were made at the appropriate season and that the rosette leaves are not commonly included on the specimens. Lesions resembling those produced by downy mildew, however, occurred on *N. longiflora*, collected on March 3, 1934, near Raymondville (Willacy Co.) and on *N. glauca*, collected on February 9, 1942, near La Joya (Hidalgo Co.). Although these specimens were examined microscopically the cause of the lesions remains in doubt.

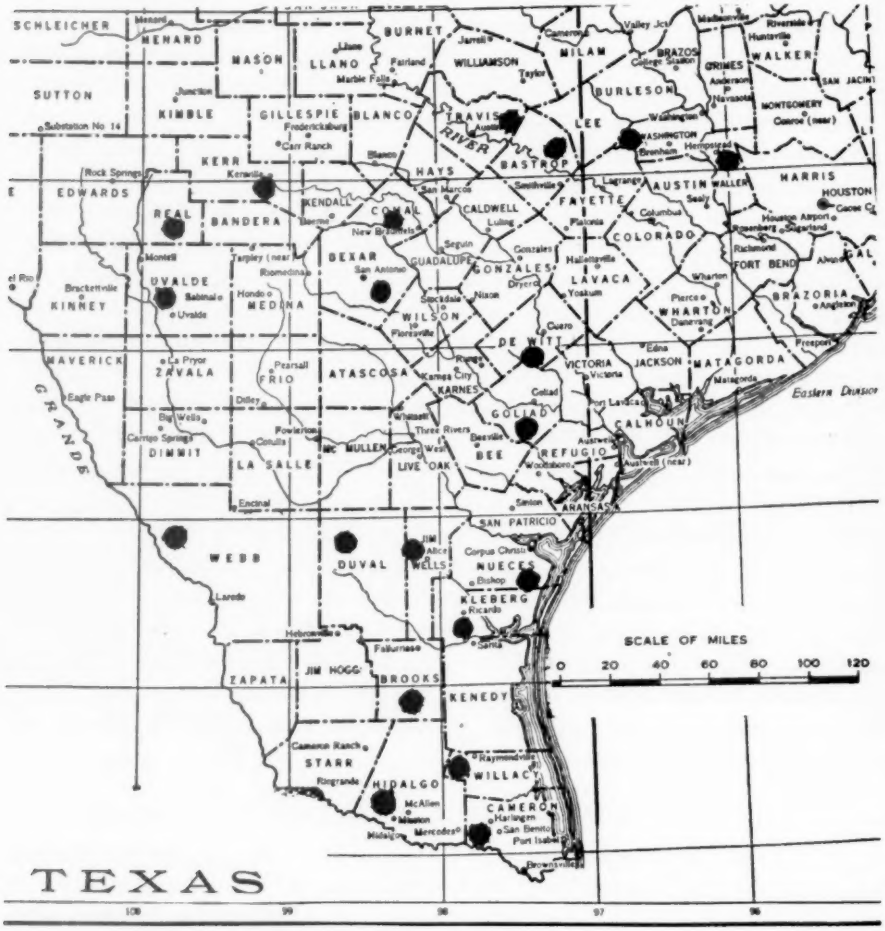

FIG. 1. Range of *Nicotiana repanda* in southern Texas. One or more specimens have been collected in each county indicated.

Present observations indicate that *Nicotiana repanda* is one of the most common and abundant weeds in citrus groves and truck gardens in the lower Rio Grande Valley, there being at times thousands of plants per acre. Plants of all ages were found, varying from young ones in the rosette stage

to those bearing flowers and mature seedpods. The flower stalks varied in height from a few inches to six or more feet, dependent upon moisture and nutrition.

Within the lower Rio Grande Valley infection by downy mildew was universal. When viewed from above, young lesions appeared as yellow, indefinite-margined areas; the lower surface of such lesions was loosely covered with a "turf" of sporangiophores. Older lesions consisted of large necrotic areas, irregularly circular, brown, and one to two centimeters in diameter. The lower surface of such lesions was usually covered with a dense grayish coating of sporangiophores. During favorable weather the

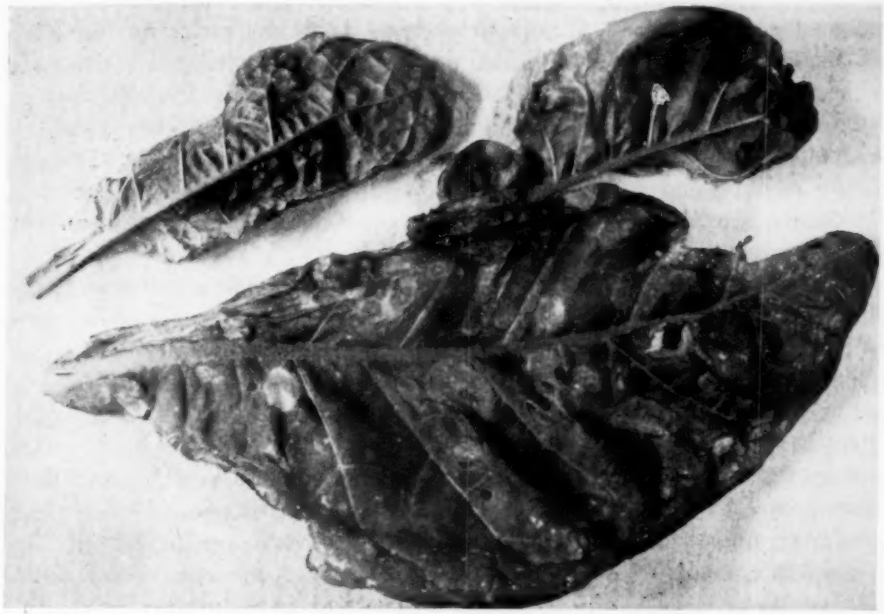

FIG. 2. Downy mildew lesions on leaves of *Nicotiana repanda*. Large necrotic lesions are produced and the leaves are deformed.

lesions enlarge by extension of the mycelium and fresh crops of sporangia were produced on this marginal zone of newly invaded tissue. Large portions of the leaf surface become necrotic and dry by confluence of lesions. As a consequence of unequal expansion of the uninvaded portions, the leaves may become curled, distorted or otherwise deformed (Fig. 2).

Lesions occurred most abundantly on the rosette leaves or other basal leaves near-by, some of which were entirely dead and decaying. When tissues from such leaves were rendered translucent by placing them in a solution of lactic acid and were examined microscopically, the old necrotic tissues were found to contain an abundance of oospores, that are structurally indistinguishable from oospores of *Personospora tabacina* occurring in decaying leaves of cultivated tobacco. Moreover their range in size accords with that of the oospores of *P. tabacina*.

Germination of sporangia was attempted since it was apparent that fresh crops of sporangia were at hand. The sporangia were suspended in tap water and kept in an icebox for approximately three hours. On being examined after this period, it was observed that germ tubes had been formed having a length of 10 to 20 times that of the sporangium.

DISCUSSION

It is now difficult or perhaps quite impossible to determine definitely the region and range within which each of the different species of *Nicotiana* is endemic. Man has unintentionally extended the range of certain of them, as indicated by Goodspeed (9), who states that the seed and capsules adhere to the coats of such animals as sheep and goats and are thus scattered locally. *N. glauca* and *N. accuminata*, for example, have become widely dispersed throughout the world. Goodspeed (9) believes the former species is native to the foothills of the Andes Mountains in northwestern Argentina, but it also has been found to occur in Australia, Egypt, the Cape Verde Islands, and has several times been introduced, with ballast, into California, Florida, and New Jersey. Moreover it has now become abundant along the lower Rio Grande.

All available evidence indicates that *Nicotiana repanda* is native to Texas and to the area in Mexico contiguous to the lower Rio Grande. It seems entirely reasonable to assume, from the observations that have been recounted, that *Peronospora tabacina* is also endemic on *N. repanda* throughout this entire region. Here as a consequence of utilization of the land for citrus and truck growing, made possible by irrigation and the use of commercial fertilizers, *N. repanda* has increased in abundance and its growth is much more luxuriant than in adjacent uncultivated areas. Under these conditions of agricultural development *P. tabacina* has coincidentally increased in amount. Moreover the severity of attack on such "cultivated" *N. repanda* has also greatly increased. An extensive reservoir of inoculum, having an area of approximately 2000 square miles, has thus been created in the lower Rio Grande Valley alone.

The survival of *Peronospora tabacina* from one season to the next, as with many other Peronosporaceae, is known to be accomplished by oospores. Apparently oospores of *P. tabacina* have not been reported previously in any wild species of tobacco, but they are produced in abundance in decaying leaves just as in cultivated tobacco. There seems no doubt that such oospores in *Nicotiana repanda* are responsible for survival of downy mildew, during summer and fall in the Rio Grande region.

It is well established that *Peronospora tabacina* is not limited to species of *Nicotiana*. The writer observed it in the Rio Grande Valley on mature plants of currant tomato, *Lycopersicon pimpinellifolium* Mill. It has been reported by various observers to occur on seedlings of tomato, *Lycopersicon esculentum* Mill.; pepper, *Capsicum annuum* L.; and eggplant, *Solanum melongena* L. Wherever *P. tabacina* occurs on these related sus-

cepts, however, the primary inoculum seems to have been derived from *Nicotiana*. The survival of this fungus for extended periods in any region is almost certainly dependent upon the existence there of species of *Nicotiana*, either native or cultivated.

Attention may be directed to the fact that nowhere in Texas is tobacco a commercial crop, although from time to time small plantings were made in the southern and eastern parts of the State. As previously mentioned, the sporangial stage of a downy mildew was collected on cultivated tobacco grown near Hallettsville, in 1906. It seems highly probable that this pathogen came from native tobacco growing near-by. Prior authentic records of endemism of *Peronospora tabacina* in southern Texas are lacking. The writer was informed, however, by Mr. H. M. Taylor, Falfurrias, Texas, that in 1892, while making a survey in the lower Rio Grande region, he noticed large brown lesions on leaves of *Nicotiana repanda*. It now seems highly probable that these lesions were induced by *P. tabacina*.

The writer (14) has previously stressed the desirability of collecting oosporic material on *Nicotiana* in the West as an aid toward solution of the problem of endemism of *Peronospora tabacina* in North America. Why this downy mildew did not earlier bridge the gap between the West and the East remains speculative, as stated by Clayton and Stevenson (5), but such speculations now have increased interest because of certain additional facts and correlated observations. A highly probable explanation of the recent introduction of downy mildew into the East is apparently provided by three interdependent circumstances. (1) Along with the agricultural development in the lower Rio Grande Valley, that first attained large proportions in the period from 1910 to 1920, there occurred coincidentally an enormous increase in abundance of *N. repanda* and its parasite, *P. tabacina*. (2) The planting of tobacco in the Georgia-Florida area was initiated in the period 1910 to 1920. As a direct consequence of the increased use of cigarettes, following World War I, and increased demand for leaf tobacco for their manufacture, there was thereafter a large increase in the acreage devoted to tobacco culture in this region. (3) Sporangia of *P. tabacina* are dispersed by air currents. Epidemiological evidence abundantly demonstrates that sporangia may be disseminated over long distances. Such evidence is supported by direct proof (17) involving the use of devices to entrap sporangia at considerable distances from their source.

With the existence of abundant inoculum in southern Texas, the provision of tobacco seedbeds in Georgia and Florida to serve as an essential stepping-stone between Texas and the contiguous tobacco-growing areas in South Carolina, North Carolina, and Virginia, it remains only to be proved that actual transportation of sporangia over this route occurred. Such proof is not directly available. The writer believes this occurred, however, in 1921 and 1931 and can be expected to recur whenever sporangia are abundant coincidentally with protracted periods of favorable temperature, relative humidity, and cloudiness. An examination of records on file at the

U. S. Weather Bureau Station, Houston, Texas, shows that favorable periods of three to five days duration are normally frequent during January, February, and March. Prevailing surface winds are from the north and northeast although the winds aloft are prevailingly toward the northeast, a circumstance that supports the present hypothesis.

The range of *Peronospora tabacina* on native species of *Nicotiana* in Texas and Mexico is so extensive that its eradication must be regarded as impracticable. Even if it were possible to eradicate the parasite on cultivated tobacco in critical areas in the East, the likelihood of reintroduction must be considered as an ever-present threat.

A plant geographer may well raise the puzzling question as to whether *Peronospora tabacina* is to be regarded as endemic both to Australia and to North and South America, since these land masses are separated by such vast expanses of ocean. Analogous situations are not rare however among fungi. *Urnula geaster* Peck, for example, is known to occur only in Texas and in Japan; *Sarcoscypha minuscula* Boud. and Torrend, in Portugal, Bermuda, and California; *Poria cocos* Wolf, in the southeastern United States and China; and *Ophionectria cylindrothecia* Seaver, in Ohio and Bermuda. No plausible explanation of these occurrences can be offered at this time and observations such as these are difficult to harmonize with generally accepted tenets of evolution. As regards *P. tabacina* the writer is inclined to the belief that it originated in the Americas and was introduced into Australia from the Americas.

SUMMARY

This study involves the endemism and identity of a fungus causing the downy-mildew disease of cultivated tobacco.

On several native species of *Nicotiana* in Texas and Mexico, especially *N. repanda*, there occurs a downy mildew having sporangia and oospores identical with those of *Peronospora tabacina* from cultivated tobacco. All available evidence indicates that *P. tabacina* is endemic to southern Texas and the contiguous part of northern Mexico.

A reasonable explanation for the original outbreaks of downy mildew on cultivated tobacco in the East is based upon three circumstances: (1) The utilization of the lower Rio Grande region for citrus and truck crops has favored the abundance of *Nicotiana repanda* as a weed and coincidentally the growth of its parasite; (2) The planting of tobacco and the increase in acreage devoted to its cultivation in the Georgia-Florida area have provided a stepping stone in the progression of the pathogen from native tobacco in the West to cultivated tobacco in the East; (3) The organism in its sporangial stage is air-borne.

Eradication of *Peronospora tabacina* does not appear feasible or practicable and such a venture would probably result in failure.

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CHLAMYDOSPORE GERMINATION AND ARTIFICIAL CULTURE OF USTILAGO STRIIFORMIS FROM TIMOTHY AND BLUEGRASS

M. J. Thirumalachar and James G. Dickson

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The stripe smut caused by *Ustilago striiformis* (West.) Niessl occurs on numerous grasses and is of economic importance on bluegrass, *Poa pratensis* L. (5), timothy, *Phleum pratensis* L., and red top, *Agrostis gigantea* Roth. Failure to germinate or differences in type of germination have been reported for the chlamydospores of this fungus during the past eighty years (2). The more recent investigations (2, 3, 4, 7) have indicated considerable variation in the fungus and physiologic specialization on the grasses. Preliminary investigations by the writers have demonstrated a technique for spore germination, the wide diversity in type of development found in this species in artificial culture, and the production of chlamydospores in the several isolates from timothy comparable to those described by Leach et al (6) from bluegrass.

CHLAMYDOSPORE GERMINATION

Good germination of fresh spores was obtained with the method described by the senior author (8). Glass slides with a dried smear of spores were inverted on a rack one inch above a water surface and wet paper toweling was placed over the backside of the slides. Counts on the maximum germination were made after 2 to 10 days. In some instances individual germinating spores were transferred to potato-dextrose agar or to water agar for further study and culture; in other instances the mass of germinating spores was dispersed in melted agar and poured into Petri dishes. The spores collected in the autumn from smutted timothy and bluegrass plants in the field and placed on slides, as described, started germinating after 5 days at 65° to 72° F. and from 30 to 70 per cent had germinated after 10 days. Spores from the sori covered by the epidermis germinated 2 days earlier and with higher percentages than spores from the older sori. Fresh spores collected from smutted plants in the field in the spring or from plants in the greenhouse showed about the same high germination percentages as those collected in the autumn.

The type of spore germination was investigated in some detail, since reports (1, 3, 4, 7) have indicated a range from branching promycelial to sporidial development, with indications that the various types of germination represented variations in the biotypes of the fungus as well as in the effects of environment. Davis (1), Kreitlow (4), and Leach et al (7) described the germination of chlamydospores by the formation of branching promycelia, although Davis noticed some sporidiumlike cells. Fischer (3) reported *Ustilago striiformis* forma *hordei* as predominantly a sporidium-

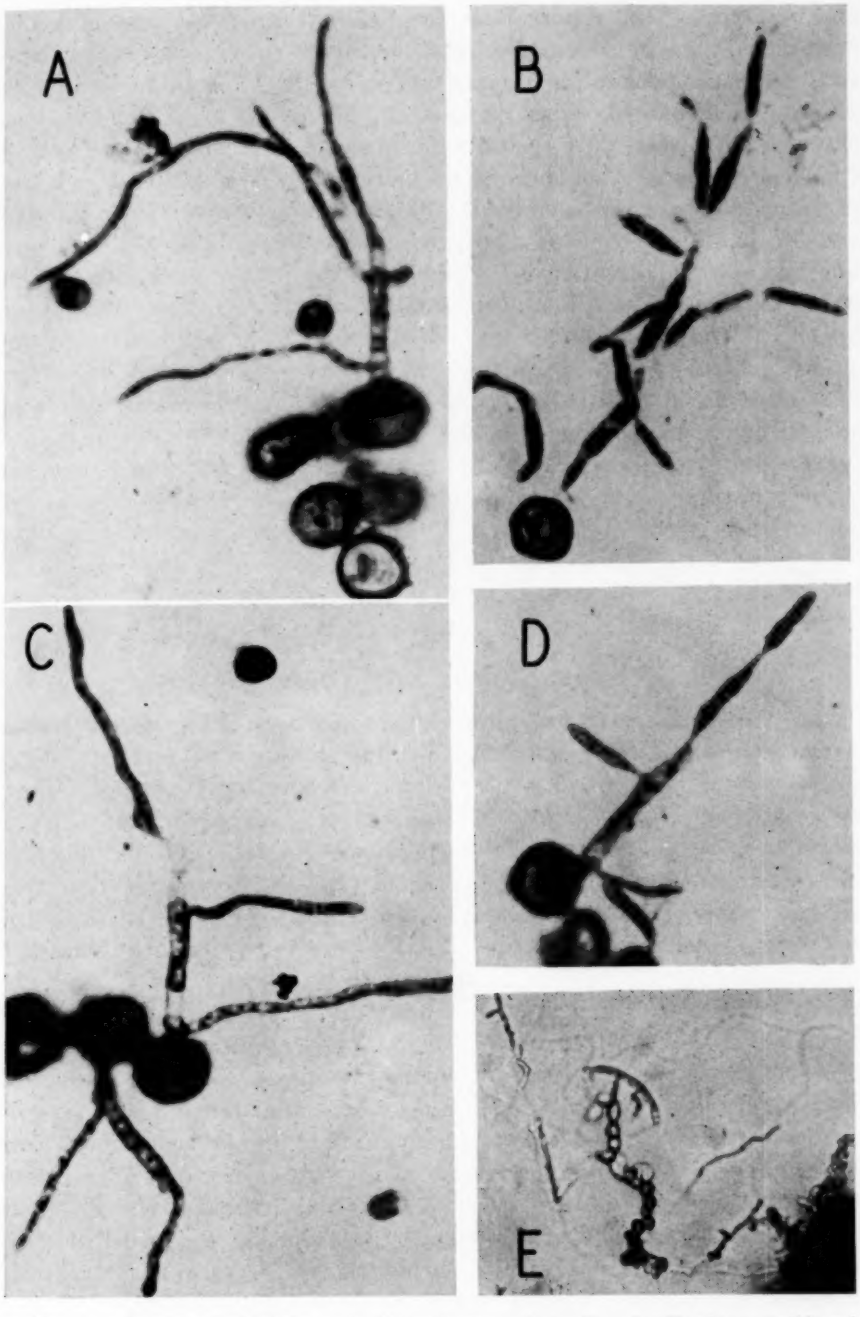

FIG. 1. Spore germination and chlamydospore formation in *Ustilago striiformis*. A. Germinating chlamydospore from timothy showing branching promycelium. ×1000. B. Same with lateral and terminal sporidia. ×1000. C. Germinating chlamydospore from bluegrass showing branched promycelium. ×1000. D. Same with sporidia. ×1000. E. Radial strands of hyphae on potato-dextrose agar, some of them rounding off into chlamydospores. ×150.

forming type. The writers have secured both sporidial and branching promycelial types of germination from the fungus on bluegrass and timothy. In the investigations in the autumn on spores from these hosts, some of the promycelia elongated either indefinitely or as much as 400 μ without branches, and others developed 2 to 3 septa and short branches (Fig. 1, A, C). The development of sporidia was not evident in these first experiments in which moisture was abundant. In germination experiments in which some of the slides were exposed to semi-dry conditions after spore germination had started, the promycelia developed septa followed by constriction of the promycelial cells into sporidiumlike structures. This was followed by lateral and terminal budding of secondary sporidia. Following this observation, individual spores were transferred to 5 per cent water agar soon after germination had started. After 4 days the promycelia fragmented and sporidia were abundant (Fig. 1, B, D). The germination of fresh spores from timothy the following spring indicated the same response to low moisture. Spores germinating in water on slides were dried after 8 days or when germination had started and were replaced in the germination chamber on 3 successive days. Later, 80 per cent of the spores so treated had produced sporidia. All isolates, however, did not show this response to drying and aeration.

CULTURE OF THE FUNGUS ON MEDIA

The fungus has been grown on culture media in either its sporidial or mycelial forms. Fischer (3) reported culturing a sporidial form of *Ustilago striiformis* forma *hordei;* Kreitlow (4) reported mycelial colonies of forma *agrostidis;* and Leach et al (6) described the complete cycle including chlamydospore formation in forma *poae-pratensis.* The latter described two distinct types of colonies: a mycelial type and a mycelial type fragmenting into sporidialike masses. Certain of the old cultures of both types produced ovate chlamydospores. The writers have obtained isolates of several types from both timothy and bluegrass. A sporidial type was obtained from spores from bluegrass that developed masses of secondary sporidia. On certain media the sporidial forms tended to produce sectors of the mycelial type (Fig. 2, B). Further investigations on the response of physiologic races are in progress. Spores from timothy produced mycelial cultures of several types as well as sporidial types (Fig. 1). The fragmenting-mycelial type produced a fine, densely interwoven mat of mycelium. Along the periphery of the colony, finely coiled hyphae abstricted small, oidiumlike [sporidiumlike according to Leach et al (6)] cells which on separation developed new colonies. Chlamydospores were formed in many of the older colonies. Thicker hyphae, easily differentiated from the fine, interwoven mycelium, developed radially with protoplasmic contents near the apices followed soon by abundant, close septations. Some hyphae produced short branches which in turn formed septa. The cells of these hyphae soon became ovate, enlarged, and thicker-walled; they finally developed into

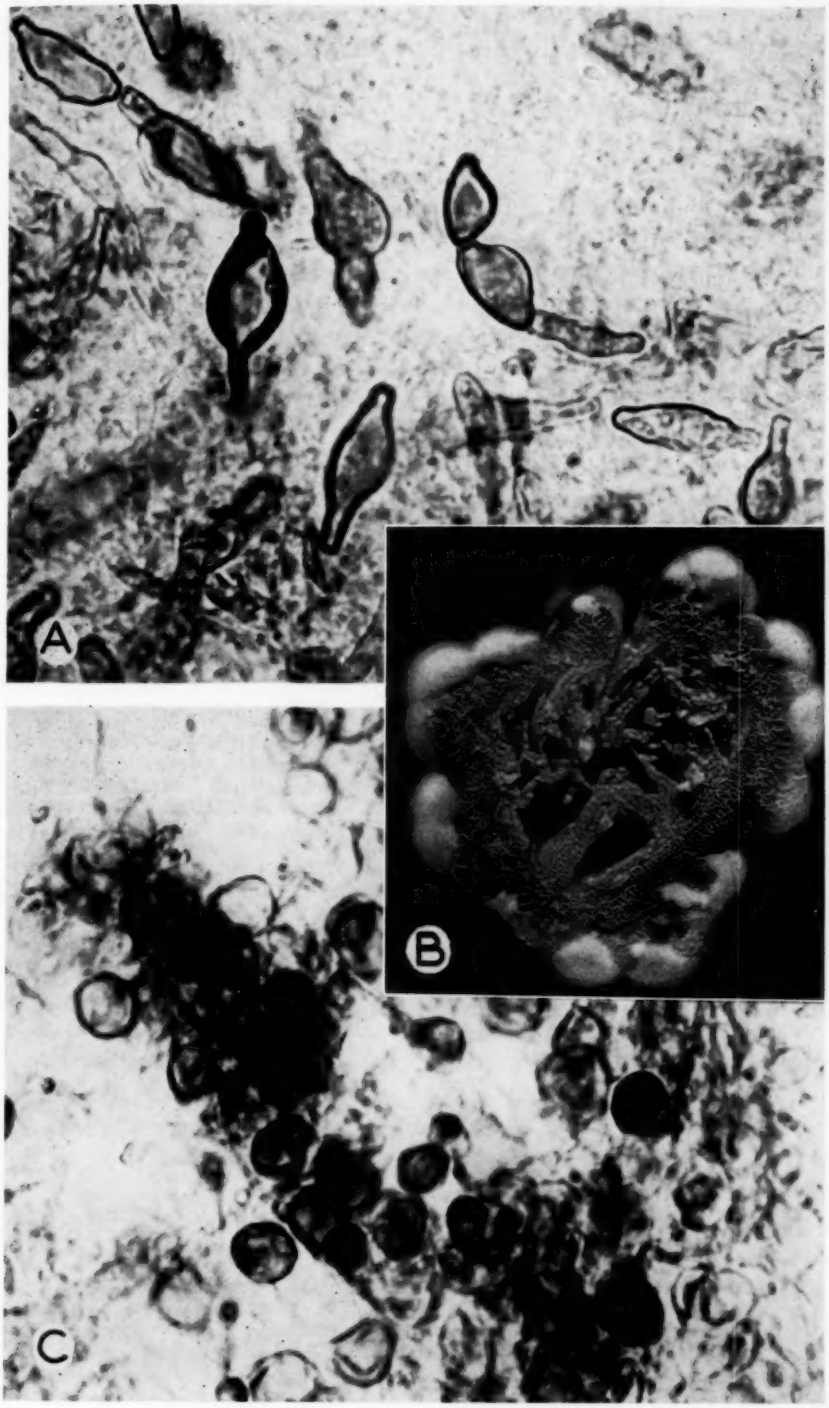

FIG. 2. Chlamydospore development and sporidial-mycelial colony of *Ustilago striiformis*. A. Ovate to lemon-shaped chlamydospores produced in artificial culture of timothy smut. ×1000. B. Sporidial colony of spores from bluegrass showing mycelial sectors along the periphery. ×1½. C. Spherical chlamydospores developed in artificial culture of timothy smut. ×1000.

mature spores (Fig. 1, E). The chlamydospores were usually ovate or lemon-shaped (Fig. 2, A), although one isolate produced spherical spores (Fig. 2, C). All had less marked echinulations and slightly greater size than the spores produced in host tissue. The mycelial type, described by Leach et al (7), and several other types have been isolated from the spores from timothy. Some of these remained vegetative; others produced chlamydospores in abundance. In these, small, yellowish-brown, short hyphae developed close septations; the cells became ovate; the walls thickened. The lemon-shaped spores remained attached or separated.

Good spore germination was obtained in fresh spores of *Ustilago striiformis* from bluegrass and timothy. Sporidial and mycelial types were obtained in artificial culture by single spore, sporidial, or mycelial isolations. Chlamydospore development in culture was observed in the several cultural types.

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WOUND INFECTION OF OAK TREES WITH CHALARA QUERCINA AND ITS DISTRIBUTION WITHIN THE HOST[1]

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INTRODUCTION

Oak wilt has continued to encroach upon the oak population of the Upper Mississippi Valley (1, 2, 3, 6) since this disease and its causal agent, *Chalara quercina* Henry, were described (4, 5).

The introduction of the fungus into a host stem resulted in the disease, and the same organism was recovered from roots, stems, branches, twigs, and leaves of infected trees (5). These and other host-parasite relations have needed clarification as a basis for efforts to combat the disease.

This paper describes studies on different parts of the tree as infection courts and on the distribution of the pathogen within the host during disease development.

MATERIALS AND METHODS

Studies on different parts of the host as infection courts for the pathogen were made on black oak (*Quercus velutina* Lam.) woodland trees at 3 locations in Wisconsin and on red oak (*Q. borealis* Michx. f.) seedlings in the greenhouse at Madison, Wisconsin. Some bur oak (*Q. macrocarpa* Michx.) and white oak (*Q. alba* L.) woodland trees were stem-inoculated. There was no oak wilt within $\frac{1}{4}$ mile of the field plots. The trees ranged in size from greenhouse seedlings and woodland seedlings and sprouts 18-inches high to 6-inch (d.b.h.) trees 30 feet tall in their natural habitat.

The woodland trees were inoculated in the roots, stems, branches, twigs, or leaves during the period of June 16 to July 13, 1943, and results were obtained by early September, 1943. The greenhouse seedlings were inoculated in roots, stems, or leaves during April, 1942 or 1943, and the data were recorded later each summer. Root inoculations were made after scraping the soil from around a desired lateral root (tap root in the case of seedlings), and after washing the root surface with distilled water followed by 95 per cent alcohol. A culture of the pathogen on cornmeal was placed in a diagonal knife cut in the vascular tissue. The wound was covered with moist absorbent cotton and the soil was replaced. In some cases the pathogen was mixed with the soil around a tree and the roots were wounded or left unwounded. Stem and branch inoculations were

[1] In cooperation with the Wisconsin Conservation Department. The assistance of E. L. Ball, J. X. Thompson, and A. Troemner during various stages of this investigation is gratefully acknowledged. The figures were prepared by Eugene Herrling.

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made in a cut made with a chisel through at least two annual rings (5). One root, stem, or branch inoculation was made per tree. Twig, petiole, and leaf-blade inoculations were made by holding the selected part for one minute in a spore suspension and stabbing it in two or three places with a sharp, sterilized scalpel (dissecting needle in the case of leaf blades). The leaf midribs of some seedlings were injected in two places with a spore suspension. One or two twigs, petioles, or leaf blades, respectively, were inoculated per tree.

Four isolates of *Chalara quercina* were used to inoculate the woodland trees. Two of the four (C and E) were the same isolates used previously (5), except for an additional passage through the host. Isolate C, a single-spore culture originally from black oak, was used for all the leaf-blade, petiole and twig inoculations and for some root and stem inoculations on black oak. Isolate E, a single-spore culture originally from bur oak, was used for stem inoculations on black, bur, and white oaks and for some root inoculations on black oak. Isolate I, a mass culture from bur oak, and isolate J, a mass culture from red oak, were used for stem inoculations on black and bur oaks. Isolates A, C, D, E, F, G, and H (5), plus isolate I, two isolates from black oak, and one from red oak were used to inoculate seedlings in the greenhouse.

An inoculated tree was considered to be infected when foliage symptoms developed, and *Chalara quercina* was reisolated from a branch.

The distribution of the pathogen was studied within naturally diseased and stem-wound-inoculated woodland trees that showed symptoms in 1941, 1942, or 1943. The 38 naturally diseased trees, from various locations in Wisconsin, were all black and red oaks except 1 tree and 1 sprout clump of white oak. They were from 1 to 22 inches d.b.h. and 10 to 70 feet high. Twenty-six of them were used to study the vertical distribution of *Chalara quercina*, 1 of the 26 plus 9 others to study the distribution in the leaves, and 3 additional trees, all red oak, to study radial distribution in the stems. The 27 stem-wound-inoculated trees were all black oaks of sprout origin in a plot near Madison and ranged from 1 to 3½ inches d.b.h. and 11 to 25 feet high. Six were used to study the vertical distribution of *C. quercina*, 1 of the 6 plus 2 others to study radial distribution in the stems, 1 of the 6 plus 4 others to study distribution in the leaves, and 1 of the 6 plus 15 others to study distribution in relation to foliage symptoms. The presence of the pathogen in a particular part of an infected tree was determined by isolation according to described methods (5).

WOUNDS AS INFECTION COURTS FOR CHALARA QUERCINA

Inoculation of Woodland Trees. Different parts of 173 black oak, woodland trees were wound-inoculated with *Chalara quercina* to determine some of the possible portals of entry for the pathogen. Some stem-inoculated bur and white oaks were included in the plots. The 3 geographical locations, the size of the trees, and the 4 isolates of *C. quercina* had no apparent effects on the results. A summary is given in table 1.

The time between inoculation and the first-noticed symptoms varied extensively between individual trees. It was influenced perhaps by the place of inoculation. The incubation periods according to the place of inoculation were: root, 21 to 48 days; ground level, 55 to 82 days; breast height, 19 to 50 days (for bur oak, 49 to 51 days); branch, 26 to 55 days; twig, 17 to 48 days; petiole, 48 to 70 days; and for the one positive case of leaf blade inoculation, 55 days. There was some error in recording the longer incubation periods; it was impractical daily to observe widely scattered trees; thus symptoms were well advanced in some cases when first noted. The shorter periods (17 to 26 days) were fairly accurate; symptoms were noted when only a few leaves had wilted.

Characteristic symptoms developed regardless of where the trees were inoculated. The first symptoms on those inoculated in the roots or stems

TABLE 1.—*Results from wound-inoculating different parts of woodland oak trees with Chalara quercina*

Host species	Part of tree inoculated						
	Root	Stem		Branch	Twig	Leaf	
		Ground level	Breast height			Petiole	Blade
Black	8/3[a]	7/4	123/46	8/3	8/8	6/4	13/1
Bur	.....	.....	30/5	.....	.....	.....	.....
White	.....	.....	5/0	.....	.....	.....	.....
Controls (black)							
Wounded, uninoculated	2/0	.....	4/0	1/0	.....	3/0	.....
Unwounded, inoculated	3/0	.....	.....	.....	6/0	6/0	6/0

[a] Numerator is the number of trees inoculated in the respective part. Denominator is the number of trees which developed symptoms and from which *C. quercina* was isolated.

appeared on scattered branches in the upper half of the crown. On the branch-, twig-, and leaf-inoculated trees, the first symptoms appeared, respectively, on the inoculated part. The symptoms soon spread throughout the crown in all cases. Branches were collected during this "intermediate" symptom stage for reisolation of the causal fungus. The collections usually were made as far away from the inoculation point as possible, particularly of branch, twig, and leaf inoculations. The terminal leader was taken in several cases. *Chalara quercina* was isolated from all inoculated trees which showed symptoms, except one stem-inoculated black oak.

The extent of symptom development by September 2 to 14, 1943, when the last notes were taken, varied between individual trees; there was little, if any, relation to the point of inoculation. At least 70 days had elapsed since all the root-, branch-, twig-, and leaf-inoculated trees and many of the stem-inoculated ones had been treated. With the exception of 2 ground-level-inoculated, 2 petiole-inoculated, and some of those stem-inoculated in July, all positive trees had shown symptoms at least 30 days previously. From

50 to 100 per cent of the primary leaves on trees of this group were dead, and from 0 to 98 per cent had fallen. Secondary leaves on suckers along the stem and main branches appeared on only 1 (root-inoculated) tree. Those trees on which symptoms had not been noted prior to the last observation date were, of course, less severely affected.

Wounds into the wood or vascular tissues of roots, stems, branches, twigs, or leaves of black oak evidently served as infection courts for *Chalara quercina*. The number of trees used in all except the breast-height studies was relatively small; thus, the relative effectiveness of the different infection courts must be considered accordingly. However, the twig inoculations appeared the most effective since 100 per cent of such treated trees became diseased. Leaf petiole was the next most effective infection court with 66 per cent positive cases. Ground-level inoculations were slightly less effective with 57 per cent; root, breast-height, and branch inoculations were equally good with 37 per cent, and leaf-blade inoculations were the least effective with 8 per cent positive results. Perhaps the penetration of the vascular elements by the causal fungus was more nearly assured by the method used for twig and petiole inoculations than by that for root, stem, and branch inoculations. Why the stem inoculations in this series of trials were only about one-half as successful as those previously reported (5) remains unexplained.

Comparatively few positive cases of disease resulted from inoculations on bur oak. The results were completely negative on white oak; this indicates further their relative tolerance (5).

None of the unwounded-inoculated or wounded-uninoculated controls became diseased.

Inoculation in the Greenhouse. In conjunction with the field studies on infection courts for *Chalara quercina,* 123 red oak seedlings growing in the greenhouse were inoculated into the roots, stems, or leaves. The results were as follows: of 11 unwounded seedlings in infested soil, 0 was infected; of 9 with wounded roots in infested soil, 0 was infected; of 15 wound-inoculated in the roots, 4 were infected; of 70 wound-inoculated in the stem, 16 were infected; of 9 injected in the midrib of the leaf blade, 1 was infected; of 9 stabbed in the leaf blade, 0 was infected. None of the 17 appropriately wounded and uninoculated controls became infected. *C. quercina* isolates A, C, D, E, F, and G gave the positive results.

The symptoms developed like those on woodland-inoculated trees. The incubation period was 14 days (2 root-inoculated seedlings) to 48 days for all except the one positive leaf inoculation; it required 103 days.

The results indicated (1) that wounds in the roots, stems, or leaves of red oak seedlings could serve as infection courts for *Chalara quercina,* (2) that infection from contaminated soil was not promising, (3) that infection from root and stem inoculations was less than that obtained with woodland trees, and (4) that infection resulting from leaf inoculations was about equally poor in both instances (Table 1).

Cross Inoculation. The presence or absence of host specificity in isolates of a pathogen bears a vital relation to the epidemiology and control of a disease. Isolates of *Chalara quercina* from black, red, bur, and scarlet oaks were pathogenic to black oaks, and one isolate from black oak was pathogenic to white oak (5). Current data confirm some of these results and demonstrate that isolates from bur and red oak may infect bur oak and that isolates from bur, black, red, and scarlet oak may infect red oak seedlings. Though some isolates failed to cause infection on red oak seedlings, there was no indication of host specificity. Isolate E, from bur oak, was pathogenic to black, red, and bur oaks but did not cause infection in any of the five white oaks inoculated (Table 1). This could be a case of host specificity, but such a conclusion would not be justifiable since it is the only instance and is based on only five individuals. Though cross inoculation studies should be carried further, the preponderance of evidence to date indicates the absence of host specificity in isolates of *C. quercina.*

DISTRIBUTION OF CHALARA QUERCINA WITHIN THE HOST[4]

Vertical Distribution. The occurrence of *Chalara quercina* in the roots, stems, and branches of infected trees has been reported (5). Information was sought on the relative frequency of the pathogen's occurrence in the different parts of the host. Isolations were made from the branches and roots of 26 naturally infected red, black, and white oak and 6 inoculated black oak trees, and from the twigs and stems of some of them. All the twigs and branches used for isolations had typical foliage symptoms.

The results were as follows: For the naturally infected trees, of the 26 root isolations, 15 were positive; of the 8 stem isolations, 7 were positive; of the 26 branch isolations, 26 were positive; of the 10 twig isolations, 8 were positive; and for the inoculated trees, of the 6 root isolations, 3 were positive; of the 6 stem isolations, 6 were positive; of the 6 branch isolations, 6 were positive; and the one twig isolation was positive. Thus, the pathogen was present in the branches of all the trees tested, in the twigs and stems of nearly all, and in the roots of over one-half of them. The host species did not seem to influence the vertical distribution of the pathogen.

Root isolations from the 6 inoculated trees and from 10 (7 red and 3 black oaks) of the 26 naturally infected trees were made in late winter or early the next summer following the appearance of foliage symptoms.

Chalara quercina was obtained from the roots of 3 of the inoculated trees and 7 (5 red and 2 black oaks) of the naturally infected trees. Thus, it may overwinter in the roots of red and black oaks.

Radial Distribution in the Stems. The radial distribution of *Chalara quercina* in the stems of infected oak trees was studied by dissecting out and culturing the desired portions of wood from three naturally infected red oaks and three stem-inoculated (August, 1941) black oaks. Symptoms were

[4] The cooperation and assistance of C. S. Moses and C. Audrey Richards, Division of Forest Pathology, U. S. Department of Agriculture, in obtaining some of the results reported in this section are gratefully acknowledged.

first noted on the naturally infected trees in August and September, 1942. Isolations were made from trees Nos. 1, 2, and 3 (Table 2) on October 21, October 27, and September 3, 1942, respectively. The black oaks had partially leafed out and then completely wilted during the summer of 1942, forming no summer wood. Isolations were made about 18 inches below the inoculation point in July and August, 1942. The results of isolations from the spring and summer wood of the 1942, 1941, and 1940 annual rings of the 6 trees are given in table 2.

In naturally infected trees, *Chalara quercina* extended toward the center of the stem as far as the 2-year-old summer wood. The fungus was found to extend slightly farther, *i.e.*, to the 2-year-old spring wood, in inoculated trees. With the exception of tree No. 5, the pathogen was present in a relatively solid band of wood.

TABLE 2.—*Radial distribution of Chalara quercina in the stems of 6 oak trees that wilted in 1942*[a]

Tree no.	Type infection	Species	D.B.H. (inches)	Age and type of wood tested					
				Current year		1-year-old		2-year-old	
				Summer	Spring	Summer	Spring	Summer	Spring
1	natural	red	10[b]	0	+	+	0	0	.....
2	do	do	10[b]	+	+	+	0	0	0
3	do	do	20	.....	+	+	+	+	0
4	inoculated	black	5	.....	0	+	+	+	.....
5	do	do	3½	.....	+	0	+	0	0
6	do	do	2½	.....	+	+	+	+	+

[a] Positive isolation of *C. quercina* is indicated by a plus sign (+) and failure to isolate it is indicated by a zero (0).
[b] Estimated.

Distribution within the Leaves. Since *Chalara quercina* sometimes inhabited oak leaves (5), further studies were made on the occurrence of the pathogen in different parts of leaves both with and without symptoms.

Isolations were made from parts of 34 leaves from 10 naturally diseased red and black oaks and 5 inoculated black oaks. All leaves were picked from wilting trees except 4 symptom-leaves that were picked from the ground soon after falling—2 beneath a naturally infected tree and 2 beneath an inoculated tree. Leaves with and without symptoms and sections of bronzed and green blade tissue were used. The petioles from all leaves and portions of the midribs, of lateral veins, and of blade tissues from some of the leaves were cultured. Results were similar with leaves from red and black oaks, so the data in table 3 are not separated according to hosts.

Chalara quercina was present most often in the petioles of diseased leaves, about half as frequently in the midribs and lateral veins, and seldom in the green portions of the blades. It was not found in the bronzed portions of blade tissues. The distribution of the pathogen in a representative

TABLE 3.—*Distribution of Chalara quercina in leaves from infected red and black oak trees*

Type infection	Symptoms	Part of leaf cultured				
		Petiole	Midrib	Lateral vein	Blade	
					Green portion	Bronzed portion
Natural	progressive bronzing	20/14[a]	15/5	9/5	15/2	12/0
Do	none	5/1	4/0	2/0	4/0	.....
Inoculated	progressive bronzing	8/5	5/1	6/0	5/0	4/0
Do	none	1/1	1/0	.....	1/0	.....

[a] Numerator is the number of leaves cultured in the respective part. Denominator is the number of leaves from which *C. quercina* was isolated from the respective part.

leaf is illustrated in figure 1, B. It was isolated from the petioles but not from other parts of 2 of the 6 leaves that showed no symptoms. The petioles and no other parts of the 4 symptom-leaves taken from the ground beneath wilting trees also yielded *C. quercina.* It may be concluded that the pathogen is likely to be present in partially bronzed leaves and may be present in leaves that show no symptoms.

Distribution in Relation to Symptoms. In relation to the possibility of control of oak wilt by pruning or sanitation measures, it is pertinent

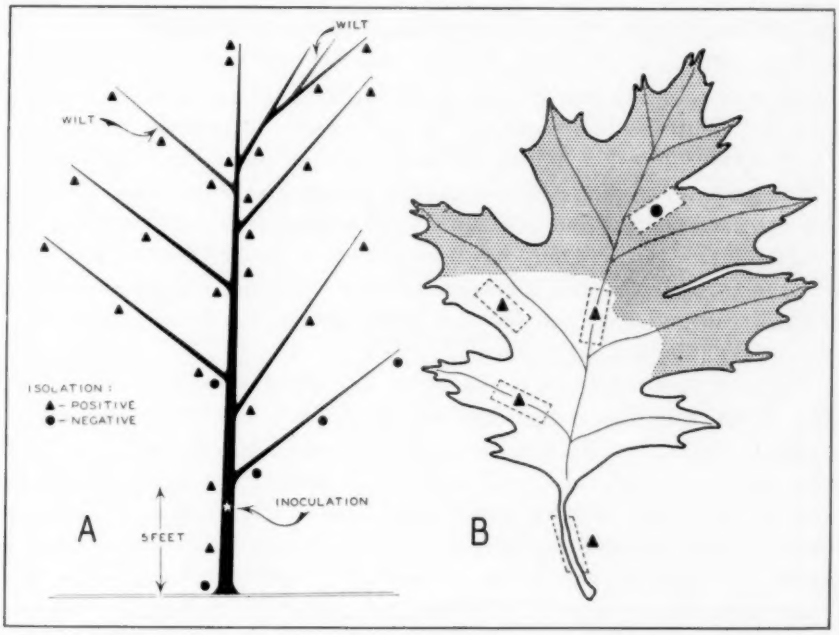

FIG. 1. Distribution of *C. quercina;* the results of isolations are indicated. A. Stem-inoculated black oak tree felled the first day symptoms were noted (24 days after inoculation). Two branches had wilted foliage; others appeared normal. B. Leaf picked from a naturally infected red oak tree; stippled, apical portion was bronzed; basal portion was green.

to know the distribution of the causal fungus in relation to symptoms. Such a study was carried out with 16 stem-inoculated black oaks. Soon after the appearance of symptoms, samples were taken from branches with wilting leaves and from other branches that appeared healthy. *Chalara quercina* was isolated from symptom and non-symptom branches of all the 16 trees.

Three of the trees were felled as soon as symptoms were noted, *i.e.*, 24, 36, and 38 days after inoculation, respectively. From 36 to 56 separate isolations were made per tree from 2 inches above the ground line to and including the current year's terminal in the apex of the crown. The distribution of *Chalara quercina* in a representative tree is shown in figure 1, A. The pathogen was isolated from the terminal shoots, most of the main branches, and the stems of each of the 3 trees. It was present in the stems to within 2 inches of the ground line in 2 of the trees and to within 39 inches in the other. The latter was felled 24 days after inoculation, or 12 days sooner than the others. Of the total 132 isolations from the 3 trees, 107 yielded *C. quercina* and 25 were sterile. The negative isolations were mostly from the lower parts of the stems. Thus, by the time symptoms were evident, *C. quercina* was well distributed throughout symptom, as well as non-symptom, branches.

DISCUSSION

These results help to clarify some of the host-parasite relations and are applicable to finding the means of disease spread and control.

Since isolates of *Chalara quercina* from one oak species can infect other species, any diseased tree is apparently a source of inoculum for neighboring oaks. White and bur oaks may be particularly important in this respect as they survive longer in a diseased condition than the red and black oaks.

The presence of the fungus in the 2-year-old wood of red and black oaks does not necessarily mean that such trees were infected 2 years before, as no infected tree in this group has been known to survive for 2 years. However, the roots of red and black oaks may be possible sources of inoculum for some time, as they are the last parts to die and have been shown to harbor the pathogen overwinter.

Wounds apparently are important for infection. Since wounds in roots, stems, branches, twigs, and leaves of black oak trees may serve as infection courts for *Chalara quercina,* one suspects, for example, insects, birds, rodents, root grafts, and tools as disseminating agents. Detached leaves may be a contributing factor since some of them harbor the pathogen. However, rain and surface water seem unpromising until evidence appears that the pathogen sporulates on the surface of infected parts. The means of dissemination is obscure but must be relatively ineffective; otherwise, such a virulent pathogen would diminish the oak population much more rapidly.

Certain common measures seem unlikely to lead to successful control.

Since rapidly growing and slowly growing large and small trees on various sites may succumb to wilt (5), control through fertilization and irrigation carries little promise. Because the fungus was found distributed throughout the crowns of infected trees, even in branches and leaves not yet showing symptoms, pruning is eliminated as a counter measure.

Sanitation measures, including the killing or removal of diseased roots, might prove valuable for control in localized areas. Dietz and Barrett (3) reported limited control over a 2-year period by sanitation methods.

SUMMARY

Typical oak wilt developed and *Chalara quercina* was reisolated from black oak woodland seedlings and sprouts and from red oak greenhouse seedlings all wound-inoculated in vascular tissues of the roots, stems, branches, twigs, and leaves.

Various isolates of the pathogen did not appear host-specific among bur, red, and black oaks.

Chalara quercina was distributed throughout the crowns of wilting trees including non-symptom branches and leaves. Roots in over one-half the trees tested were diseased.

The fungus was isolated from the current-year, 1-year-old, and 2-year-old wood of the stems of wilting trees.

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EVIDENCE OF FUSION BODIES FROM UREDIOSPORE GERM TUBES OF CEREAL RUSTS ON NUTRIENT-SOLUTION AGAR

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INTRODUCTION

When urediospores of cereal rusts were germinated on nutrient-solution agar round bodies formed on the ends of some of the germ tubes. Many of these bodies fused with neighboring hyphae and the process of fusion sometimes resulted in what appeared to be extensive anastomosing unlike anything heretofore described, so far as the writers are aware. The present paper describes the "fusion bodies," the anastomosing, and some of the factors affecting their development.

MATERIALS AND METHODS

Several different kinds of media and various concentrations of mineral salts and glucose were tested in the course of the work. A satisfactory medium contained mineral salts and glucose in the following concentrations:

M/5 Magnesium sulphate	2.5	cc.
M/2 Ammonium nitrate	0.5	cc.
M/2 Calcium nitrate	1.5	cc.
M/5 Potassium acid phosphate	1.5	cc.
M/5 Dipotassium phosphate	2.5	cc.
0.5 per cent Ferric tartrate	0.5	cc.
Glucose	50	gm.
Distilled water to make	1000	cc.
Agar	20	gm.

This medium was autoclaved for 10 minutes at 12 lb. pressure. Its reaction after autoclaving was near pH 6.0.

Urediospores of the rusts listed below were dusted by means of a camels-hair brush on the surface of freshly poured nutrient-solution agar about 2 mm. thick in small Petri dishes. They were incubated overnight in darkness at approximately 13° C.

Detailed studies were made with *Puccinia graminis tritici* Eriks. and E. Henn. race 56 and *P. triticina* Eriks. (*P. rubigo-vera tritici* (Eriks. and E. Henn.) Carleton) race 105; corroborative observations were made on *P. coronata avenae* Fraser and Led., *P. hordei* Otth (*P. simplex* (Koern.) Eriks. and E. Henn.), *P. dispersa* Eriks. and E. Henn., and *P. sorghi* Schw.

DEVELOPMENT OF FUSION BODIES AND NETWORKS

Urediospores incubated overnight in darkness on nutrient-solution agar containing 5 per cent glucose produced a profuse growth of mycelium, often

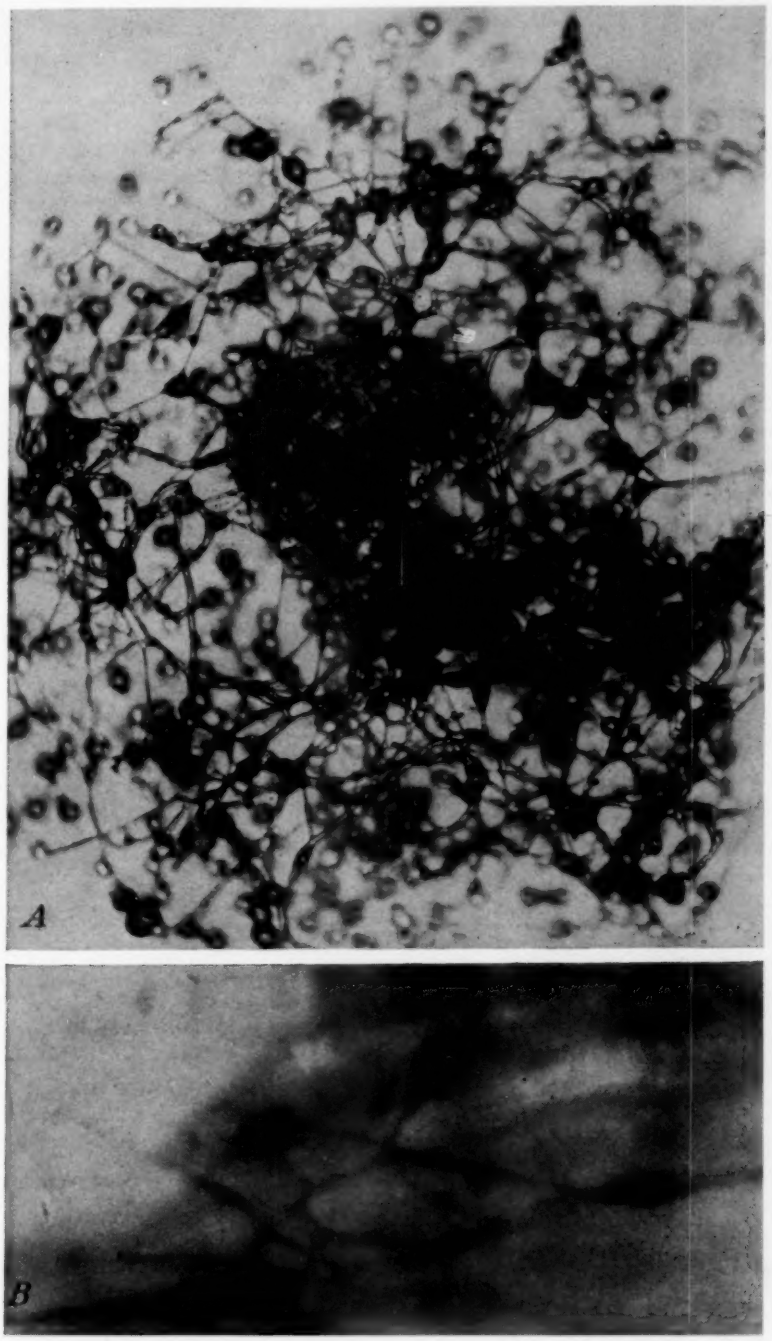

FIG. 1. Networks of hyphae of *Puccinia triticina*, with fusion bodies: A. On nutrient-solution agar; B. On surface of inoculated leaf of Little Club wheat. ×300.

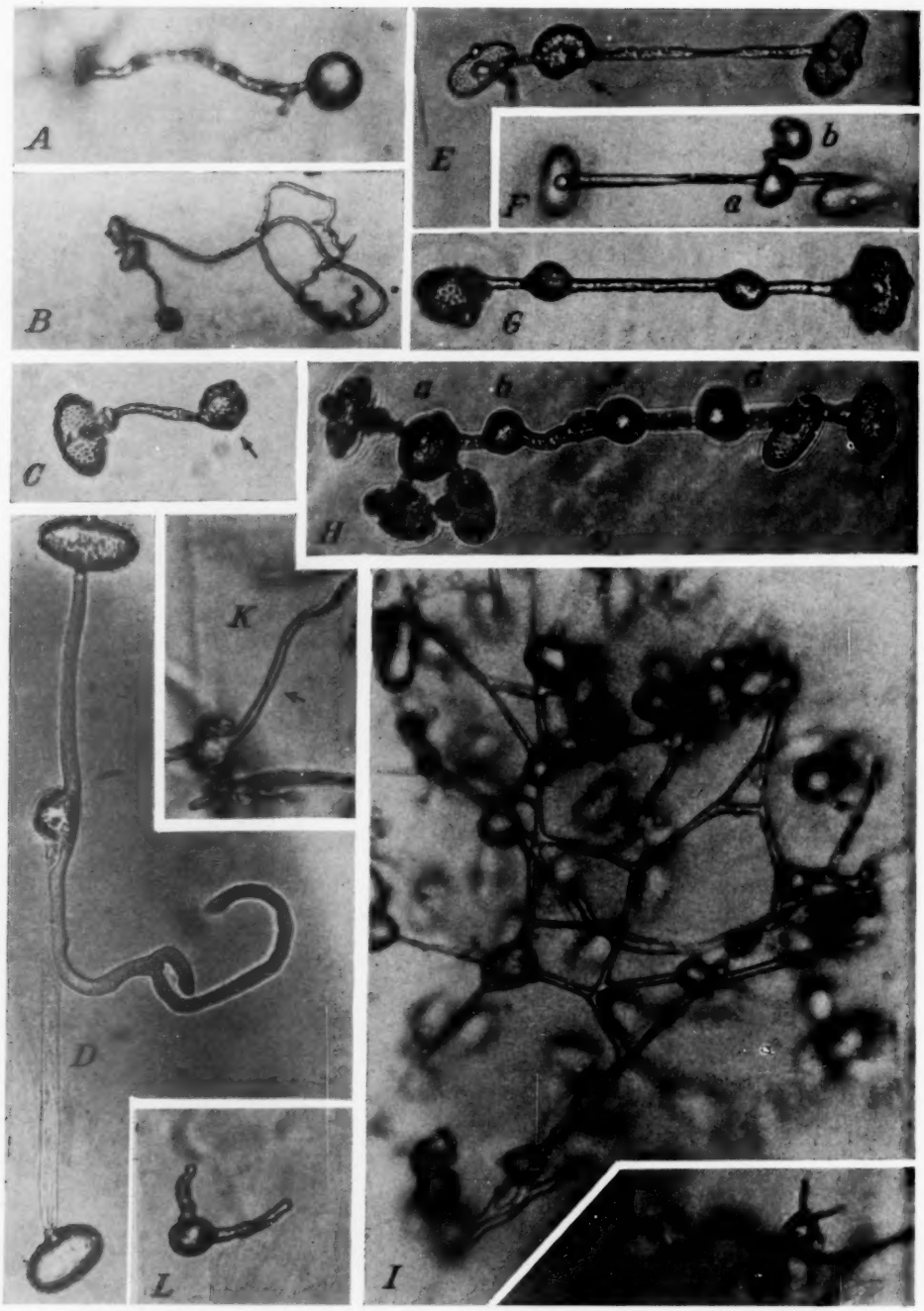

FIG. 2. Fusion bodies and hyphal network of *Puccinia graminis tritici* on nutrient-solution agar. (×440 except where otherwise noted.) A. Newly developed fusion body formed aerially on the tip of a germ tube, from a urediospore on the surface of the agar (out of focus). B. Two germinated urediospores, one of which has produced a fusion body which has been retracted toward the urediospore. ×150. C. Fusion body (arrow)

with such extensive anastomosing[1] as to form veritable networks of hyphae (Fig. 1, A). Along the strands of the hyphae were brown bodies, some almost round and others irregular in shape. The method of formation of these bodies and the networks was followed in cultures of *Puccinia graminis tritici* and *P. triticina.*

After about 5 or 6 hours' incubation the tip or lateral branch of an aerial hypha suddenly became distended to form a round body into which the contents of the germ tube were discharged (Figs. 2, A and 3, A). These bodies were about the same diameter as that of the urediospores. The suddenness with which they were formed suggested release at the hyphal tip of an internal pressure that forced the contents of the germ tube into the body. In general appearance and orange-brown color these bodies resembled those on the strands of the network (Fig. 1) except that the newly formed bodies were rounder. They are referred to herein as fusion bodies.[2]

In the usual course of events the newly formed fusion body does not remain still. After several minutes, during which the granules within it are often seen in vigorous motion, the hypha supporting it bends and the body tips downward or sideways with a slow jerking motion. If the body comes in contact with another hypha this motion is arrested and the body envelopes the contacted hypha as would a viscous drop (Figs. 2, D and 3, C). It then seems to exert a suction that gradually pulls the two hyphae taut as a stretched string. The free distal end of the contacted hypha is often suddenly pulled into the body soon after contact is made. The structure now resembles those shown in figures 2, E and 3, D. Retracted hyphae could sometimes be seen coiled within the body when the latter was broken with a micromanipulating needle soon after contact with a hypha had taken place (Fig. 3, G).

[1] This term is used without implication that intercommunication results from all contacts of fusion bodies with hyphae.

[2] Other types of bodies that formed on this medium were structures corresponding to appressoria and substomatal vesicles (7) and, rarely, teliospore-like bodies resembling those observed by Ezekiel (4).

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that has been retracted toward the urediospore. D. Fusion body, produced on a germ tube from the lower urediospore, that has contacted a germ tube from the upper spore, showing dissolution of the cell wall at the point of contact. E. Fusion body (arrow) that has made contact with a hypha whose distal end has been pulled into the body. Both germ tubes have been pulled straight. F. Like E, except the distal end of the contacted hypha has not been pulled into the fusion body, *a*, but has itself produced a fusion body, *b*, either before or soon after the contact. The germ tube producing *b* is bent at right angles by the pull from the body at *a*. G. A stage following one like F, in which the fusion body formed on the contacted hypha has fallen over and contacted the germ tube that produced the first fusion body. The hyphae between the two bodies have been drawn together and appear to be a single strand. H. Structure involving fusion bodies from germ tubes of five urediospores. The germ tube coming from the spore at the extreme right formed the fusion body at *b;* the germ tube from the adjacent spore formed the body at *d;* the germ tube from the spore at extreme left formed the body at *c;* germ tubes from the two lower spores produced bodies that coalesced and formed the large body at *a*. I. Network formed by fusion bodies contacting germ tubes and by subsequent coalescing and straightening of connecting hyphae. Urediospores producing the hyphae are on the surface of the agar below the network, therefore out of focus. × 300. J–L. Hyphae growing from fusion bodies. At K (arrow) is a portion of an unusually long hypha observed growing from a body that had fused with a germ tube. J and L, × 300.

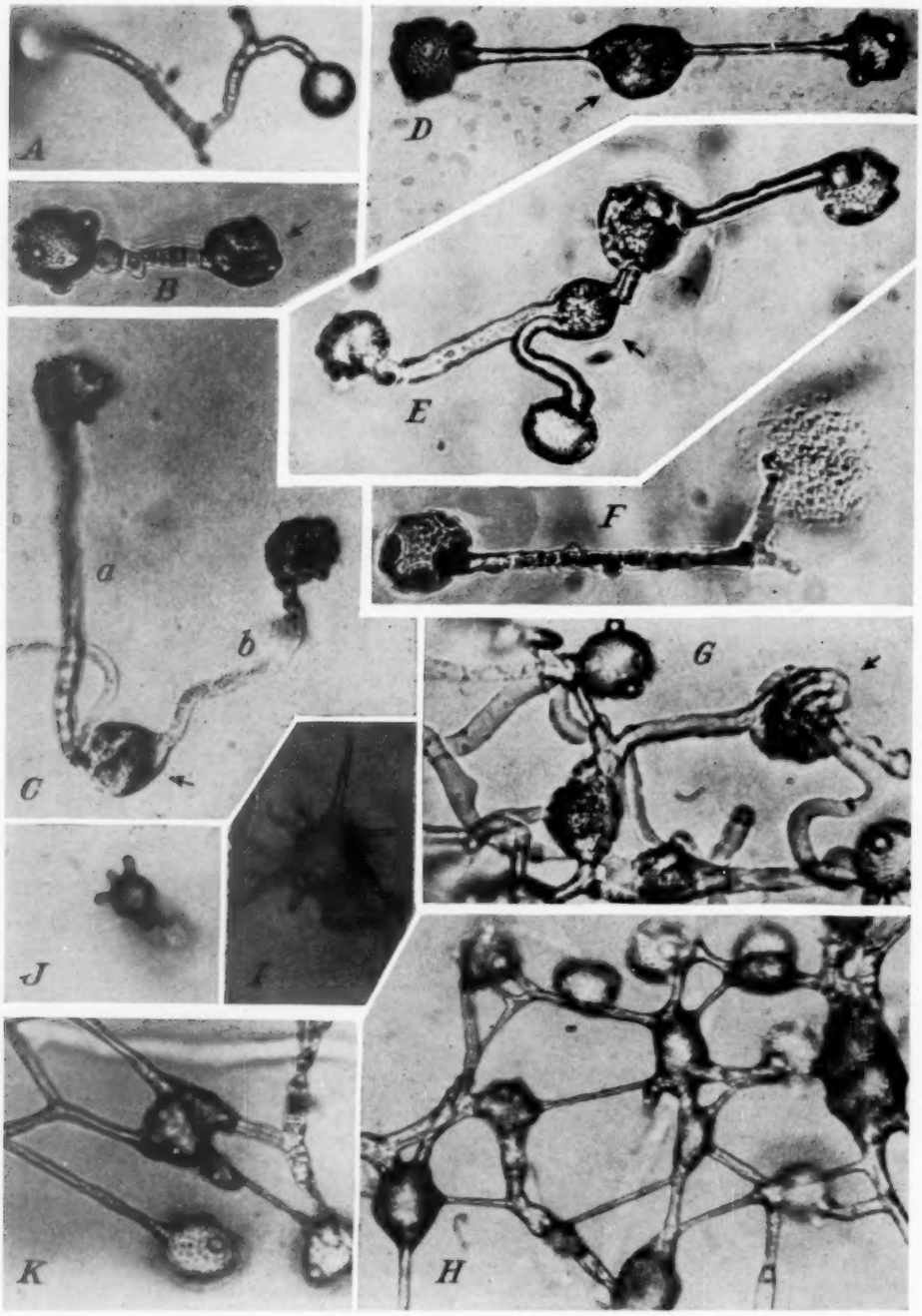

FIG. 3. Fusion bodies and hyphal network of *Puccinia triticina* on nutrient-solution agar. (×440 except where otherwise noted.) A. Newly developed fusion body formed aerially from a urediospore on the surface of the agar, out of focus. B. A fusion body (arrow) that has been retracted toward the urediospore. C. Fusion body (arrow) formed on germ tube *a*, and fused with germ tube *b*, dissolving the hyphal wall at the point of

Sometimes the distal portion of a contacted hypha is not drawn into the body. In this case it may itself form a fusion body, as illustrated in figure 2, F, *b*, which may in turn fall on and envelop the hypha that produced the original body. The two hyphae between these bodies are pulled together and thus appear as a single strand (Fig. 2, G). When additional bodies, formed on adjacent hyphae, fall on these strands, complicated structures such as that shown in figure 2, H, are produced. This structure developed as follows: (1) the fusion body at *b*, formed on the end of a germ tube from the spore farthest to the right, contacted and fused with the germ tube from the spore farthest to the left; (2) the distal end of the latter germ tube formed a fusion body, which dropped down and fused at *c* with the germ tube from the right hand spore; (3) then a germ tube from the spore adjacent to this right hand spore formed a fusion body that contacted the strand at *d;* (4) at the other end of the strand, fusion bodies from the two lower spores contacted the germ tube from the spore to the left, and, being close together, coalesced to form the large body at *a*. Even larger bodies, formed by the coalescence of several fusion bodies, were common throughout the cultures.

Fusing of many bodies with germ tubes of adjacent hyphae may result in networks such as those illustrated in figure 1, A and in the enlargements of portions of such networks in figures 2, I, and 3, H. The very large body to the right in figure 3, H, resulted from coalescence of several fusion bodies.

Many fusion bodies fail to come in contact with hyphae. In such instances, the body appears to roll back toward the urediospore, often enveloping a considerable portion of the germ tube and becoming somewhat irregular in outline owing to inclusion of the coiled hypha (Figs. 2, B and C, and 3, B). Such was the fate of the newly formed body shown in figure 3, A. It began to retract with jerky movements just before the photograph was made. Five minutes later it suddenly engulfed the outer portion of the germ tube including the nearer branch, stopping at the level of the farther branch which remained protruding. Sometimes the body envelopes all of the germ tube and comes to rest against the urediospore.

If a newly formed fusion body touches the agar it may burst or flatten

contact. Granules were observed passing from germ tube *b* into the body. D. A fusion body (arrows) that made contact with a germ tube whose distal end was then pulled into the body and both germ tubes pulled straight. E. Two fusion bodies (arrow) that are 2 days old, therefore hardened, and three urediospores, pulled out of their normal alignment by unsuccessful attempts to pull them apart with a micromanipulating needle. The structure developed as follows: (1) the larger fusion body, formed on the germ tube coming from the spore at the right, fused with the germ tube from the spore at the left; (2) the distal end of this contacted hypha was drawn into the body, resulting in a structure like that shown in D; (3) a fusion body on the germ tube from the lower spore touched and fused with the contacted hypha to the left of the first-formed fusion body. F. A fusion body broken by contact with the agar surface before hardening. G. Fusion body (arrow) broken with a micromanipulating needle, showing a coiled germ tube in the body. H. Network formed by fusion bodies contacting germ tubes and by subsequent coalescing and straightening of connecting hyphae. The very large bodies resulted from coalescing of fusion bodies. I–J. Hyphae growing from fusion bodies. Large body at I was formed by coalescence of adjacent bodies and germ tubes as in H. ×300. K. Fusion bodies and hyphal network from germinating urediospores of *Puccinia dispersa*.

out as would a drop of liquid, and the granular contents spread on the agar, as shown in figure 3, F. In some cultures this happened to many of the bodies.

Although fluid[3] in early stages, the fusion bodies harden so that by the second day they can be manipulated with a needle without bursting. For several hours after contact is made between a body and a hypha they can be pulled apart readily with a micromanipulating needle, but after hardening has occurred the strands can no longer be easily separated. Many unsuccessful attempts were made to separate the hyphae involved in such structures, one of which, after manipulation with a needle, is shown in figure 3, E.

It appeared in several instances, such as that illustrated in figure 2, D, that there is dissolution of the wall of the germ tube at the point of contact with the fusion body. Fusion of the contents of a body with that of a contacted hypha was clearly evident in the case illustrated in figure 3, C. In this instance, observations were made over a period of 2 hours and during this time some of the granular contents of the contacted hypha, *b*, were seen passing into the fusion body that had formed on the germ tube *a*. Occasionally a few granules moved in the opposite direction but the general movement was toward the body. Further opportunity for the fusion of the contents of germ tubes occurs when two or more fusion bodies come in contact with each other and coalesce, as in figure 2, H, *a*, and the very large body in figure 3, H.

For these bodies to have any significance in the life history of the rusts, or in the origin of races, they should produce hyphae containing nuclei from germ tubes of different races. Therefore, a thorough search was made for evidence of growth of hyphae from the bodies.

As a rule, examination of cultures several days old showed the fusion bodies to be somewhat shrunken but otherwise unchanged. In some cultures, however, a few of them produced hyphae. Rarely bodies that had not contacted germ tubes were seen with 2 or 3 short hyphae growing from them (Figs. 2, K and L, and 3, J), while larger bodies resulting from fusion with neighboring germ tubes or with other fusion bodies sometimes had formed as many as ten hyphae. With one exception these bodies from which hyphae emerged were near mycelia of contaminants, *Rhizopus* or *Alternaria*. The exception was a body with no contaminants nearby that produced several hyphae one of which was under observation while it grew to a length of approximately 300 μ (Fig. 2, K). In a few instances a hypha of a contaminant was seen involved in a fusion body. In such cases one could not be sure but that the hyphae protruding from the body were from the contaminant, stimulated to excessive branching perhaps by the food supply provided by the mass of protoplasmic material of the fusion body. In other cases, so far as could be seen, only rust hyphae were involved. Here it would appear that production of the hyphae by the fusion bodies was stimulated by some material or condition resulting from the presence of the contaminants.

[3] The contents of the broken bodies were sticky and adhered to the micromanipulating needle. The mass of material that accumulated thereon was soluble in ammonium hydroxide.

The question then arose as to whether fusion bodies form on the surface of leaves. Seedlings of Little Club wheat were inoculated with urediospores of *Puccinia triticina*, race 105, according to the usual procedure, and incubated overnight. The following morning there was profuse growth of rust hyphae, and occasional networks were seen with irregular dark bodies on the strands (Fig. 1, B) similar to those formed on the agar plates (Fig. 1, A). Since fusion bodies did not form on agar except when it contained certain nutrients, there was evidently exudation of nutrient material from the plant tissue. Evidence of such exudation of substances into water drops on the surface of a leaf has been reported by Brown (3) and Arens (2).

Factors Affecting Formation of Fusion Bodies

Various factors influenced the number of fusion bodies formed. In the first place, there was considerable variability in the number of bodies formed by different spore lots of the same species germinated under what seemed to be identical conditions. In the second place, it was evident that certain environmental factors determined the number of bodies obtained with any given spore lot. Detailed studies were made, therefore, to determine the effects of nutrients, temperature, and the intensity and quality of light.

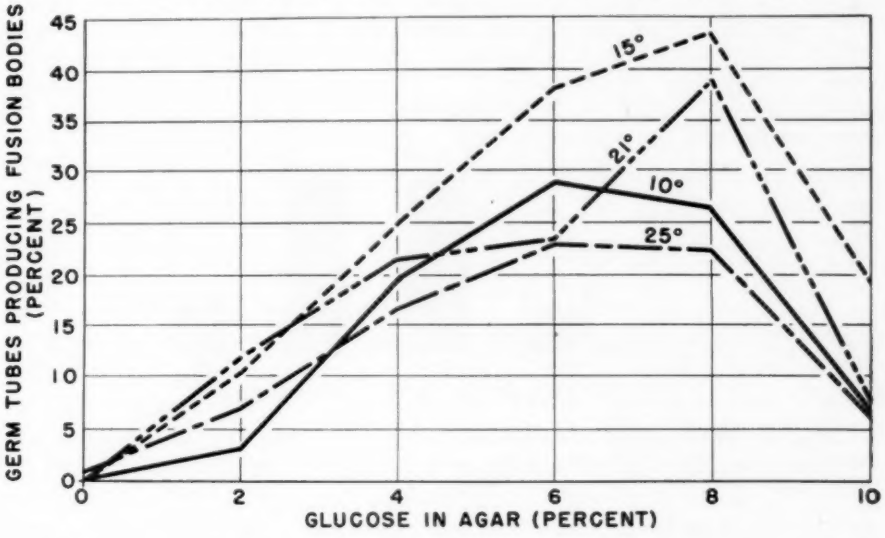

FIG. 4. Effect of glucose on the formation of fusion bodies by *Puccinia triticina* on nutrient-solution agar, incubated overnight at different temperatures (° C.).

Although good germ tube development was obtained on plain agar, almost no fusion bodies were formed unless both mineral nutrients and glucose were supplied.

Results of the first experiment to determine the number of bodies formed by *Puccinia triticina* at glucose concentrations between two and ten per cent are presented in figure 4. Percentages are based on counts of 400 germinated urediospores, except at 21° C. where 1200 were counted for each glucose concentration.

The data show that practically no bodies appeared where there was no glucose in the medium. The number increased as the glucose concentration increased, to a maximum at six to eight per cent. The number was greatly reduced at 10 per cent.

The longest germ tubes were produced where the glucose concentrations were not over 4 per cent. At concentrations of six per cent and over, reduction in hyphal development was sufficient to reduce the likelihood of a fusion body contacting a hypha, hence to reduce the extent of anastomosing. This effect is shown by the data of another experiment, presented graphically in figure 5, comprising separate counts of the bodies that contacted hyphae and

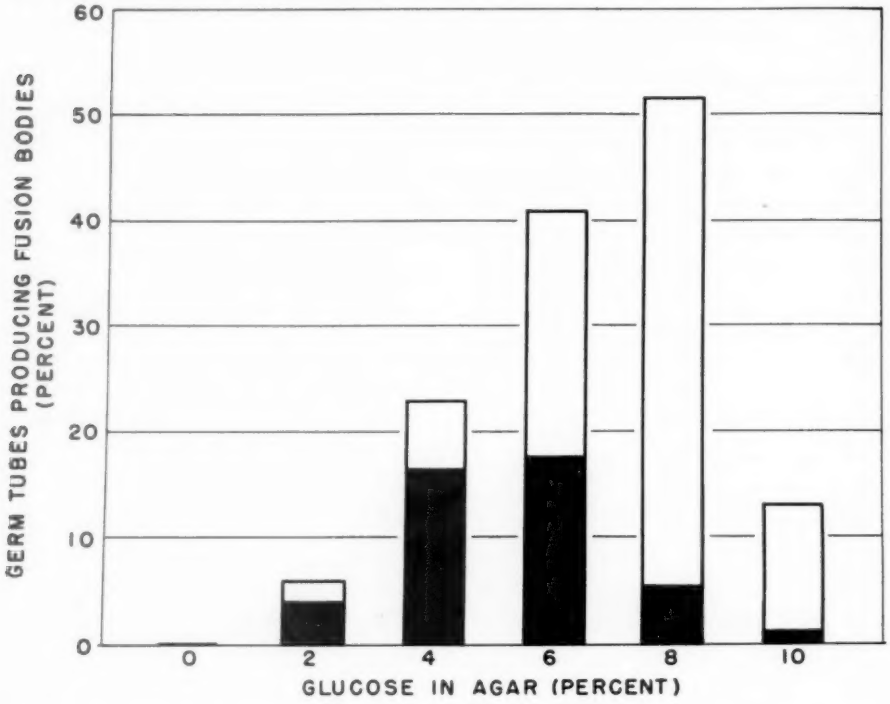

FIG. 5. Effect of glucose concentration on percentages of germ tubes producing fusion bodies. The solid parts of the bars show the percentages of germ tubes that anastomosed with neighboring germ tubes.

those that remained isolated. Urediospores of *Puccinia triticina* were germinated at 17° C. (± 1°) on agar drops suspended in Van Tiegham cells. All the drops contained the mineral constituents of the nutrient solution but differed in the concentration of glucose. Each bar of the diagram represents averages for 800 germinated urediospores, 100 counted in each of 8 cultures.

The data in figure 5 show that in the absence of glucose almost no bodies were produced. Their number increased with increasing glucose concentration up to 8 per cent, with a sharp drop in number at 10 per cent. At 2 and 4 per cent, the hyphae were long and intermingled, as when the spores are germinated on plain agar, and a relatively high proportion of the bodies con-

tacted hyphae. At the 6-per-cent level more bodies formed than at 4 per cent, but reduced growth of the germ tubes lessened the chances of contacts, so that the number of bodies fusing with hyphae was approximately the same as at 4 per cent. At the 8-per-cent level, the germ tubes were so short that a large percentage of the bodies remained isolated. At 10 per cent comparatively few bodies were formed and but a few of these made contact with other hyphae. Similar results were obtained in comparable tests with *Puccinia graminis tritici.*

As shown in figure 4, the greatest number of bodies was obtained at 15° C. at each glucose concentration except 0 and 2 per cent where relatively few bodies were formed. In other tests, however, there was very little difference over the entire range of 10° to 25° C., although usually there was a rather definite minimum at 25°. It is concluded that over the range favorable for germ tube development, temperature is not an important factor for production of fusion bodies.

Light inhibited the formation of the bodies. Urediospores of *Puccinia graminis tritici* were germinated on nutrient-solution agar in two constant-temperature rooms at 16° C., one of which was illuminated with fluorescent light having an intensity at the approximate surface of the agar of 34 foot-candles,[4] while the other was kept dark. No bodies developed in the lighted room whereas the usual number formed in the dark room. Similar results were obtained when a 200-watt Mazda lamp was used as a source of illumination.

In other experiments, urediospores of *Puccinia triticina* were germinated in natural daylight on nutrient-solution agar plates placed at different distances from a north window. No bodies developed near or even at some distance away from the window where the light intensities were 100 and 20 foot-candles, respectively. A few bodies were formed where the intensity was one foot-candle. Only in very dim light, 0.4 of a foot-candle, were there as many bodies as in complete darkness.

To determine the effect of light quality on fusion-body formation, germinating urediospores of *Puccinia triticina* were exposed to light from a 200-watt Mazda lamp transmitted through red, yellow, and blue Wratten filters, respectively. The intensity of the light near the surface of the agar was approximately 45 foot-candles behind the red and yellow filters and 10 foot-candles behind the blue one. An additional spore lot was germinated in darkness.

The data in table 1 and similar results obtained with *Puccinia graminis tritici* and *P. coronata avenae* showed that the components of white light responsible for the inhibition of fusion bodies are the shorter wave lengths at the blue end of the spectrum. Since the bodies formed normally behind the yellow filter that transmitted down to about 5000 Å, it can be concluded that the inhibiting wave lengths are those between 4000 and 5000 Å. It should be noted that the intensity of the blue light that produced the inhibit-

[4] These intensity measurements are only approximate because the Weston Illumination Meter used was standardized for sunlight.

ing effect was but a fraction of that of the red and yellow light that had no effect on body formation.

It was noted in the course of these investigations that the germ tubes grew in a direction away from the source of white light, as has been observed by others (5, 6, 8, 11). The data in table 1 show that the blue constituents of white light are responsible for this orientation, as found previously by Forbes (5). The germ tubes subjected to the light transmitted by the yellow and red filters were not oriented although this light was much stronger than that behind the blue filter.

An impression was obtained from the various experiments that the most fusion bodies were formed by relatively fresh spores. To determine the effect of age of spores, counts were made every two weeks on a spore lot of *Puccinia graminis tritici* and one of *P. triticina* of the same age stored in the refrigerator at 13° C. The spores were germinated on nutrient-solution

TABLE 1.—*Effect of red, yellow, and blue light on fusion-body formation and orientation of germ tubes in Puccinia triticina germinated on nutrient-solution agar*

Color and intensity of light				Fusion bodies	Orientation of germ tubes
		Transmission in the visible spectrum			
Filter no.	Color	Wave lengths in Angstrom units	Intensity in foot-candles[a]		
.....	Darkness	.....	0	Many	0
2412	Red	6000–7200	45	Many	0
3486	Yellow	5000–7200	44	Many	0
5543	Blue	4000–5400	10	0	+

[a] Measurements are approximate because the Weston Illumination Meter used was standardized for sunlight.

agar containing 6 per cent glucose, and incubated overnight at 13° C. For the first three months there was no significant change in the percentages of germ tubes that produced fusion bodies. The percentages ranged from 24 to 41 for stem rust and 26 to 42 for leaf rust. After three months the figures decreased, somewhat more rapidly for stem rust than for leaf rust, until after about four and a half months the percentages were all less than 10.

There was little difference between the various species of rust with respect to the number of fusion bodies formed, with the exception of *Puccinia sorghi* whose germ tubes showed such a strong tendency to grow down into the agar that relatively few of them produced bodies.

DISCUSSION

Reports of fusions of hyphae of rust fungi within the host plant have been made by Plowright (10, p. 5) and Allen (1). The former stated that mycelia ramifying within the tissues united to form an "anastomosing irregular network"; and the latter found that intercellular hyphae of *Puccinia triticina* sometimes fused, forming an "enlarged mass" at the point of union,

from which grew other hyphae. The present paper, based on work with six species of cereal rusts, presents evidence of fusion by means of special bodies on germ tubes from urediospores germinated on a nutrient-containing agar.

Fusion bodies may have been seen by Plowright (9 and 10, p. 32) on germ tubes of *Puccinia graminis* [*Uredo linearis*] from urediospores germinated on a drop of water. In describing them he said the terminal extremity of the tube became expanded in a globular manner, "into which all the yellow endochrome accumulated." He concluded that they were "endochrome reservoirs" (9) or "spores of reserve" and implied that they formed on the surface of leaves (10, p. 32).[5]

No other report of these round bodies has been found in the literature. Apical swellings were observed by Ezekiel (4) on various media and were described by him as teliospore-like. Bodies resembling them were occasionally seen in the present investigation. They were quite unlike the spherical fusion bodies.

Some networks of hyphae, with fusion bodies on the strands, were observed on the surface of wheat leaves inoculated with *Puccinia triticina*. Since a supply of mineral nutrients and glucose was essential for the development of the bodies on agar, it is assumed there was exudation of nutrient materials from the inoculated leaf tissue.

Hyphae from fusion bodies developed occasionally in agar-plate cultures of both *Puccinia triticina* and *P. graminis tritici*. The nuclear condition of these hyphae remains to be determined; and proof of the practical significance of the fusion process will depend on the results of pathogenicity studies. The observations made thus far suggest strongly that here may be a method whereby new races may arise in the absence of an alternate host. The process may account for the numerous races of such rusts as *P. triticina* in regions where the aecial stage is rarely, if ever, found.

SUMMARY

Germ tubes from urediospores of cereal rusts (*Puccinia graminis tritici, P. triticina, P. dispersa, P. coronata avenae, P. hordei, P. sorghi*) grown on agar containing mineral nutrients and glucose produced bodies that fused with neighboring germ tubes, forming anastomosed networks of hyphae above the level of the agar surface.

The bodies, designated "fusion bodies," occasionally produced several short hyphae. These hyphae formed on isolated bodies as well as on those that had fused with neighboring germ tubes. Such hyphae were seen in older cultures contaminated by *Rhizopus* and *Alternaria*, by-products from which may have had some stimulating effect on their production.

Both mineral nutrients and glucose were essential for normal production of the fusion bodies. They formed in greatest numbers on media containing 6 to 8 per cent glucose but the best concentrations for network formation

[5] Plowright refers here to work (9) in which he apparently germinated the spores on water drops, leaving doubt as to whether he actually saw the bodies on leaves.

were between 4 and 6 per cent, because of more extensive development of hyphae at these concentrations.

Fusion bodies formed at all temperatures favorable for hyphal development. They were inhibited by daylight and by artificial illumination from Mazda and fluorescent lights. This effect of light was traced to the short wave lengths at the blue end of the spectrum, between 4000 and 5000 Ångstrom units.

Hyphal networks with fusion bodies on the strands occasionally were found on wheat leaves inoculated with urediospores of *Puccinia triticina*.

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BURN BLIGHT OF JACK AND RED PINE FOLLOWING SPITTLE INSECT ATTACK[1]

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INTRODUCTION

A severe epidemic of burn blight appeared recently in Wisconsin and Michigan on jack and red pine. In 1940 MacAloney and Secrest (6) noted pines dying in lower Michigan. In 1941 dying red pines were found in northern Wisconsin. The disease was found in 1942 on both jack and red pine in Wisconsin, in Forest and Marinette Counties, respectively. Cortical browning and fungus fruit bodies were observed on killed tissue following attack by the spittle insect (*Aphrophora saratogensis* Fitch) on these two species, viz., *Pinus banksiana* Lamb. and *P. resinosa* Ait. The disease has not been reported on other tree species.

TABLE 1.—*Number of areas and approximate acreages in Wisconsin showing the burn blight disease on jack or red pine, 1945*

Counties with infection	Date of first report	Areas infected	Approximate acres involved
		Number	*Number*
Florence	1943	6	640
Forest	1941	3	120
Iron	1945	1	10
Langlade	1944	5	40
Marinette	1941	28	2600
Oconto	1943	8	2100
Oneida	1943	4	160
Price	1945	1	.....
Vilas	1943	8	780
Total	.....	64	6450

The distribution of burn blight in Wisconsin in 1945 involved approximately 6500 acres scattered through 64 areas in 9 northern counties, as indicated in table 1. It has appeared also in the Manistee Forest in lower

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Michigan and in several areas in upper Michigan. Similar heavy damage to the jack and red pine has been reported in the Cass Lake region of Minnesota.

The disease is known to be distributed over a much smaller area than is the associated spittle insect, which has been reported from Nova Scotia south to Florida, west through the Lake States and Ontario to British Columbia and California (1).

Wherever it has appeared burn blight has caused alarm. For example, the commercial lumber interests have wondered whether they will ever have the local red pine on which they were counting. The pulp and paper industries have seen ruined some of their prospects of local jack pine. The foresters have watched the dying of young stands and have questioned what can be done with the areas selected for growing these two species.

SYMPTOMS

From a distance a heavily diseased stand appears as if a fire had singed the tree tops, turning them brown and leaving the lower branches relatively green for a time. This has suggested the name "burn blight." A typically diseased tree is shown in figure 1, A. Closer examination revealed that although the tops died first, there were from few to many "flags" throughout the rest of the tree and many spittle insect punctures on living twigs.

The sequence of symptoms in a typical case on jack pine was as follows. Before any foliage discoloration appeared, insect punctures were visible at the cambium when the bark was peeled away (Fig. 1, C, I, and J). The insect feeding punctures shown in figure 1, C were tiny, light tan flecks, sometimes surrounded by infected spots that were dark, somewhat watersoaked, and coalesced as they enlarged. The subsequent spreading necrosis shown in figure 1, D, E, and H was a typical sign of fungus invasion associated with yellowing and browning of the twigs. The necrosis soon completely girdled the branch and advanced both up and down. When the fungus was growing rapidly the needles often turned brown next to the twig before they died at the outer end. The usual pattern was for the disease to start in the twigs in the upper part of the tree and to work toward and down the main stem. Occasionally the necrotic margin passed noninfected branch whorls. The twigs on these whorls then changed from green to yellow and brown. The disease has been observed at times to progress from affected side branches, which it killed, to the main stem and then to spread up and down through the bark and cambium from that point.

These symptoms have been attributed to desiccation both because of the removal of liquid by spittle insects and because of the resin blocks in the wood. Since the symptoms of burn blight and desiccation needed clarification, the progressive appearances of twigs dying from lack of moisture were examined.

Portions of jack pine trees were dried with varying degrees of rapidity. Branches removed from the tree and dried rapidly in the laboratory showed

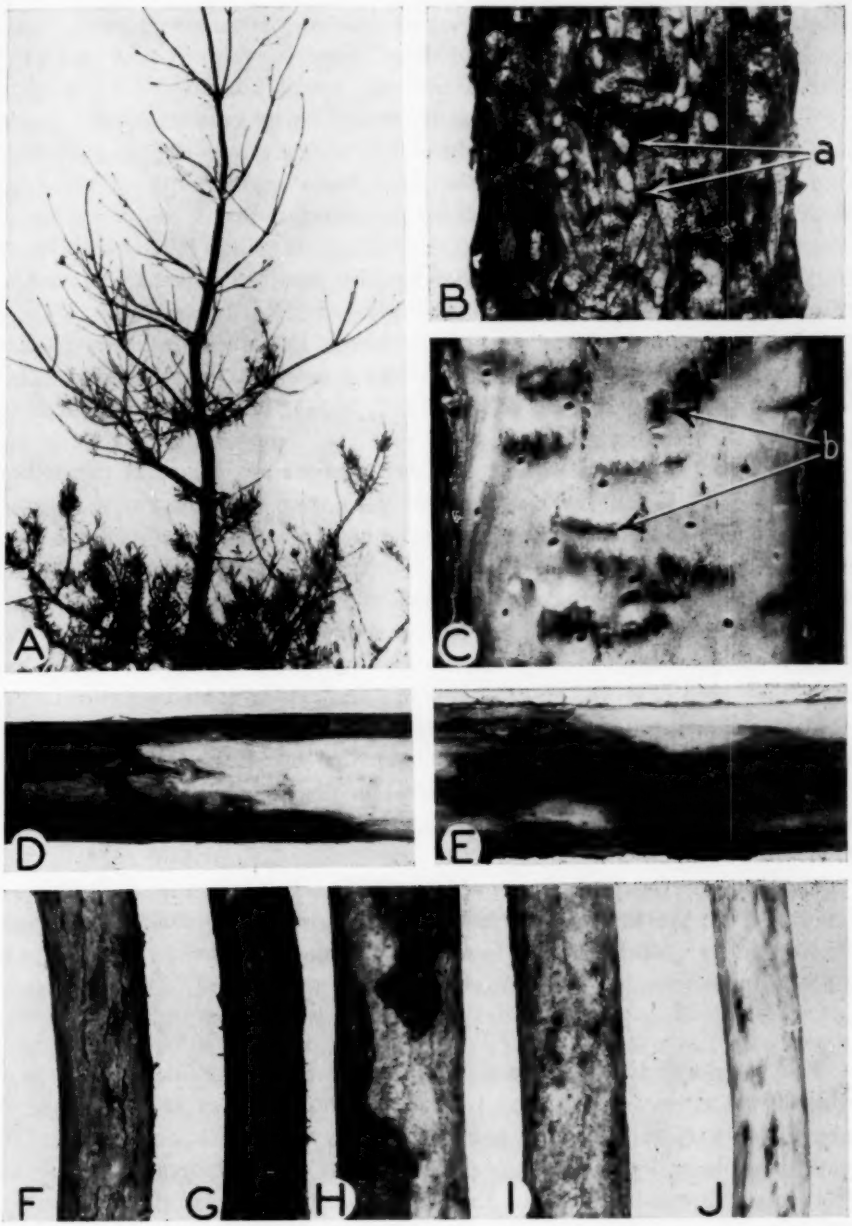

FIG. 1. A. Burn blight has killed the top of this 10-foot jack pine. B. *Chilonectria* fruiting (a). C. Brown necrotic tissue (b) surrounding spittle insect feeding punctures (red pine). D. Relatively sharp margin between dead and healthy underbark tissue. E. Coalescing fungus-invaded areas. F. Branch killed by desiccation is tan without sharp margin. G. Branch killed by *Chilonectria* is chocolate brown. H. Advanced necrotic margins. I and J. Insect injury in cambial region and in wood, respectively.

little change in the color of the cortical tissues. Branches injured on the tree by cuts of varying depths and dried slowly developed a tan and dry cortex with no sharp margin between the living and dead tissues (Fig. 1, F). Symptoms of desiccation on trees injured by cuts on the main stem corresponded with those for cut branches on the tree. In no case were fungus fruit bodies observed on tissues in these experiments. Such dried material was easily differentiated from the chocolate-brown and moist tissues newly invaded by the fungus (Fig. 1, G), tissues which had characteristic sharp margins between infected and healthy portions (Fig. 1, H) and on which reddish brown perithecia soon developed (Fig. 1, B, a).

Small orange to brown, erumpent perithecia appeared on diseased twigs, branches, and stems in as short a time as 4 weeks after infection. They were readily visible on jack pine (Fig. 1, B, a), but were frequently obscured by the bark scales on red pine.

The disease symptoms on red pine were similar but varied in two important ways: (1) Whenever jack and red pine were found growing together the incidence of insect punctures was higher on the red pine. (2) In spite of this, the disease progressed more slowly on the red pine. These two facts suggested that in the mixed stands red pine was more tasteful to the spittle insects than the jack pine and that the red pine was more resistant to the fungus. After heavy infestations of Saratoga spittle insect, red pines have been observed to recover more frequently than jack pines.

Diseased jack pine branches were sectioned and stained with Pianeze III, B. The fungus had a prolific, much-branched mycelium that invaded the cortex, killed the cambium, and penetrated to a depth of 8 or 9 cells into the xylem. The fungus frequently killed the twigs or stems by girdling them.

The twigs were killed first, perhaps because they were more frequently punctured by the insects and more rapidly girdled by coalescing fungus lesions than large branches. However, on some 12-foot trees the bark and cambium were invaded progressively down to the ground.

CAUSAL AGENT

The fungus fruiting on jack pine was identified by M. P. Backus as *Chilonectria cucurbitula* (Curr.) Sacc. Although from external appearance this organism resembles a true *Nectria,* the formation of secondary ascospores places it in the genus *Chilonectria.* These spores are small and often so numerous in the ascus that they appear as a granular mass which is easily misinterpreted. Descriptions are given by Saccardo (7) and Ellis and Everhart (2). So far as the writers know, this is the first time *C. cucurbitula* has been associated with a serious disease; therefore, its pathogenicity was examined critically.

Pathogenicity

The association of *Chilonectria* with this disease was first noted in the fall of 1943 when typical fruiting bodies occurred on almost every killed

branch. Fruiting bodies were found in 1945 on approximately 1000 affected branches.

In the fall of 1943 cultures were made from more than 40 diseased pine twigs. *Chilonectria* predominated in cultures made from the advancing brown margin in the cambium and cortex, and *Pullularia pullulans* DeB. appeared commonly from the bluish-black stains in the vascular tissue.

The culture technique employed in 1944 and 1945 was adapted from that used by Henry et al (4). The twig or branch was stripped of needles and was dipped momentarily in 95 per cent alcohol. The excess alcohol was shaken off and the remainder burned away. The outer bark was removed with sterile scalpels and about 10 chips were cut from the region of most recent discoloration and were transferred aseptically to 2 per cent malt agar in Petri dishes. After 3 days at room temperature the fungus commonly appeared as a light-colored, delicate mycelial growth, closely appressed to the agar, and radiating from the chip for a distance of about 3 to 5 mm. After 6 days the growth was more dense near the chip but was still delicate at the margin. By that time a series of concentric zones was apparent. After 7 to 10 days the surface of the growth was covered by hyaline microconidia which were orange in mass. The mycelium seldom became fluffy, and then only at high temperatures (32° to 36° C.). Final readings were made after about 7 to 10 days on all the chips. The growth on agar was characteristic and not easily confused with common contaminants.

In plantings made from the margin of cortical lesions, *Chilonectria cucurbitula* grew from all 49 jack pines and 7 red pines cultured in 1944 and from the 80 jack pines and 27 red pines cultured in 1945.

Plantings were also made from 40 healthy jack pines and 20 healthy red pines. *Chilonectria cucurbitula* was not isolated from any of these healthy trees. Among the 700 necrotic areas[4] around insect punctures observed in 1944 and 1945, 6 and 13 per cent, respectively, were infected. Evidence is presented later that the insects carried the fungus, at least externally.

Several inoculation procedures were followed during 1944 and 1945, in which a number of types of wounds were employed. Inoculations attempted without wounds all gave negative results. The wounds effective in providing a portal of entry were: needle punctures with a side-flow hypodermic needle (5), multiple punctures by an inoculator made with a large number of entomology mounting pins stuck close together through a piece of soft wood, and bruises made with a small hammer as used with *Hypoxylon* (3). Spore suspensions in distilled water were commonly sprayed on the two latter types of wounds. However, in some cases the punctures were made under spore suspensions. The multiple punctures resembled more closely the inoculations associated with the insect injuries and were used most frequently. With a large number of punctures the necrotic spots coalesced more rapidly with a correspondingly reduced time for girdling and flagging.

[4] The cambium around a spittle insect puncture showed a slight discoloration often 1 or 2 mm. across, in which the cells appeared dead.

Spores for the inoculations came either from recently killed jack pine branches or from cultures from infected jack pine tissue. Spore suspensions for inocula were prepared from three sources: (1) For natural spore material, twigs with abundant fruiting bodies were immersed about a day in distilled water. The twigs were removed and the spore suspension containing a mixture of ascospores and conidia was poured through cheesecloth to remove the debris. The resulting spore suspension was about 99 per cent *Chilonectria*. (2) Isolations from the advanced necrotic margins yielded two isolates which were purified by monoconidial transfers and were used as stock cultures. (3) Fruiting bodies on jack pine were crushed in sterile water and eight single ascospores were picked and grown as stock cultures. In the last two cases the cultures formed orange to pink colored masses of conidia, which were suspended in water for the inoculations.

The results of inoculations, showing the repeated fulfilling of Koch's postulates, are summarized in table 2.

The symptoms that followed inoculation of red and jack pines corresponded closely with those in nature.

The results from the various inoculations may be summarized as follows: At Phelps, Wisconsin, only 3 of the 120 control trees receiving only punctures developed any infection. This would doubtless have been higher if the tests had been conducted in an area where the fungus spores were abundant. No external disease symptoms were noted on the 10 trees inoculated with *Pullularia pullulans*, although this fungus stained the wood. Of the 250 trees inoculated with *Chilonectria*, 248 developed typical cortical symptoms. Of 110 left long enough, every one developed typical flagging. *Chilonectria* was reisolated from 108 of these trees.

In the greenhouse at Madison, Wisconsin, 60 jack pine trees receiving only punctures developed no disease symptoms. Among the 120 trees inoculated through punctures with *Chilonectria*, 116 developed cortical symptoms and flagging, and *Chilonectria* was reisolated from all.

In field plots at Madison, where jack pines were growing vigorously on good soil, but where there was neither spittle insect nor *Chilonectria*, 90 twigs punctured but not inoculated developed no *Chilonectria* lesions. Of 58 such twigs inoculated with *C. cucurbitula*, cortical symptoms and flagging developed on 46. The fungus was reisolated from 37 of the 46. The fungus had little tendency to spread down the twigs from the inoculation punctures, probably because of tree vigor.

The disease developed in 410 cases of the 428 inoculations made. In 261 cases out of 272 trials in which *Chilonectria* was inoculated into jack pine twigs, typical symptoms developed and the causal fungus was recovered.

Although *Chilonectria* thus appears as a pathogen, which may operate independently through wounds, the spittle insect seems important for natural development of burn blight.

TABLE 2.—*Summary of inoculations in 1944 and 1945 of jack pine with spores of Chilonectria cucurbitula*

Plot location, treatment, and origin of spores	Trees treated	Date of treatment	Trees with:		*Chilonectria* reisolated
			Disease in the fall	Flagging the next summer	
	No.		*Per cent*	*Per cent*	*Per cent*
Phelps, Wis. (in woods)					
Punctured, not inoculated	20	Aug. 23, 1944	0	0	0
Do	40	June 19, 1945	2	0	2
Do	20	June 20, 1945	0	0	0
Do	10	June 25, 1945	0	0	0
Do	10	June 29, 1945	0	0	0
Do	20	Aug. 17, 1945	0	0	0
Punctured, inoculated with spores from:					
Perithecia	20	Aug. 23, 1944	100	100	100
Lesion culture No. 1	20	Aug. 23, 1944	100	100	100
Do No. 2	40	June 19, 1945	100	100	100
Do No. 2	10	June 20, 1945	100	100	100
Do No. 2	10	June 25, 1945	100	100	100
Do No. 2	10	Aug. 17, 1945	80	100	80
Do No. 3	60	June 29, 1945	100	42	....[a]
Single-spore culture No. 1	10	Aug. 17, 1945	100	20	....[a]
Do No. 2	10	Aug. 17, 1945	100	50	....[a]
Do No. 3	10	Aug. 17, 1945	100	0	....[a]
Do No. 4	10	Aug. 17, 1945	100	30	....[a]
Do No. 5	10	Aug. 17, 1945	100	80	....[a]
Do No. 6	10	Aug. 17, 1945	100	40	....[a]
Do No. 7	10	Aug. 17, 1945	100	80	....[a]
Do No. 8	10	Aug. 17, 1945	100	60	....[a]
			Disease 6 weeks after inoculation	Flagging 10 weeks after inoculation	
Madison, Wis. (in greenhouse)[b]					
Punctured, not inoculated	60	Feb. 10, 1945	0	0	0
Punctured, inoculated with spores from:					
Lesion culture No. 4	60	Feb. 10, 1945	98	98	100
Do No. 5	60	Feb. 10, 1945	95	95	100
Madison, Wis. (in nursery)					
Punctured, not inoculated	90	June 15 to July 17, 1945	0	0	0
Punctured, inoculated with spores from perithecia	58	June 15 to July 17, 1945	79	79	80

[a] The cortical symptoms were characteristic and no reisolations were attempted.

[b] A parallel series was run on 180 red pines with 100 and 97 per cent showing disease, respectively, for culture Nos. 4 and 5. *Chilonectria* was recovered from every diseased seedling.

RELATION OF THE SPITTLE INSECT TO THE DISEASE

The Saratoga spittle insect, which is associated with the disease, has been found in the Lake States for many years. However, it was not reported as causing damage in pine stands until 1940 when MacAloney and Secrest (6) and Secrest (8) observed injury in lower Michigan.

The association of the spittle insect with burn blight was apparent not only from the frequent presence of the adult insects on diseased trees, but also from an examination of feeding punctures on several thousand trees throughout the various areas involved. Feeding by this insect preceded the discoloration of the twigs. However, death of the twigs often occurred only after 8 to 10 months. Occasionally, however, browned areas infected with *Chilonectria* were found surrounding holes made by other insects, *e.g.*, pitch mass borers, or developing near wounds made by deer, rabbits, or other agencies.

In the Phelps area the nymphal and instar stages of the spittle insect developed at the crown of various low-growing plants during May, June, and early July in 1944 and 1945. The adults appeared in early July and were active through July, August, and September. Their feeding on pine was heaviest on 1-year-old twigs and progressively less on current-year twigs, or on 2-, 3-, and 4-year-old twigs. There was little if any feeding upon branches or stems over 8 years old or over 1 inch in diameter. The fungus, however, spread into branches and stems from 1 to 14 years old.

Enormous numbers of spores of *Chilonectria* exuded whenever the mature perithecia were moistened, so spores were common on the foliage of the jack pine where they might be picked up by crawling insects.

Various stages of 85 spittle insects were collected and each was touched lightly about 20 times to 20 ml. sterile 2 per cent malt agar in Petri dishes. No fungus developed from the nymphs, and from only one of the fourth and fifth instars. However, *Chilonectria* was obtained from all bodies and mouth parts of adults used in 1944 and 1945. The obvious association of the fungus with the adult insects suggested further work on them as possible vectors.

Necrotic areas surrounding 400 insect punctures were examined in jack pine specimens from three northern Wisconsin regions. The total number of necrotic areas surrounding insect punctures and the number of such areas that contained the fungus were counted and recorded as shown in figure 2. Except in recent infections, the infected areas were larger and darker than noninfected discolored areas around punctures. Here a correlation appears between the number of necrotic areas per square centimeter and the number of such areas that were infected. The ratio of infections per square centimeter increased with an increase in the number of feeding punctures per square centimeter, as shown by the upward trends of the curves. The ratio of infected spots per square centimeter to number of necrotic areas was greater in 1945 than in 1944, possibly owing to the larger amount of fungus inoculum available as the epidemic increased.

Experiments to determine the relative parts played by the fungus and by the insects in the initiation of the disease were conducted in 1945 near Phelps, Wisconsin, on a 20-tree jack pine plot with 5 trees in each treatment. The trees were from 4 to 5 feet high and were growing vigorously in an area well removed from heavily infected trees. The trees were punctured

at the rate of 15 punctures per square centimeter along the top 2 feet of the main stem and the associated side branches. Sleeve-type wire cages 8 inches in diameter and 2 feet long with attached cloth sleeve ends were used to enclose the upper parts of the trees.

Fifteen puncture wounds per square centimeter did not produce the disease symptoms. The fungus introduced by spore suspension or by

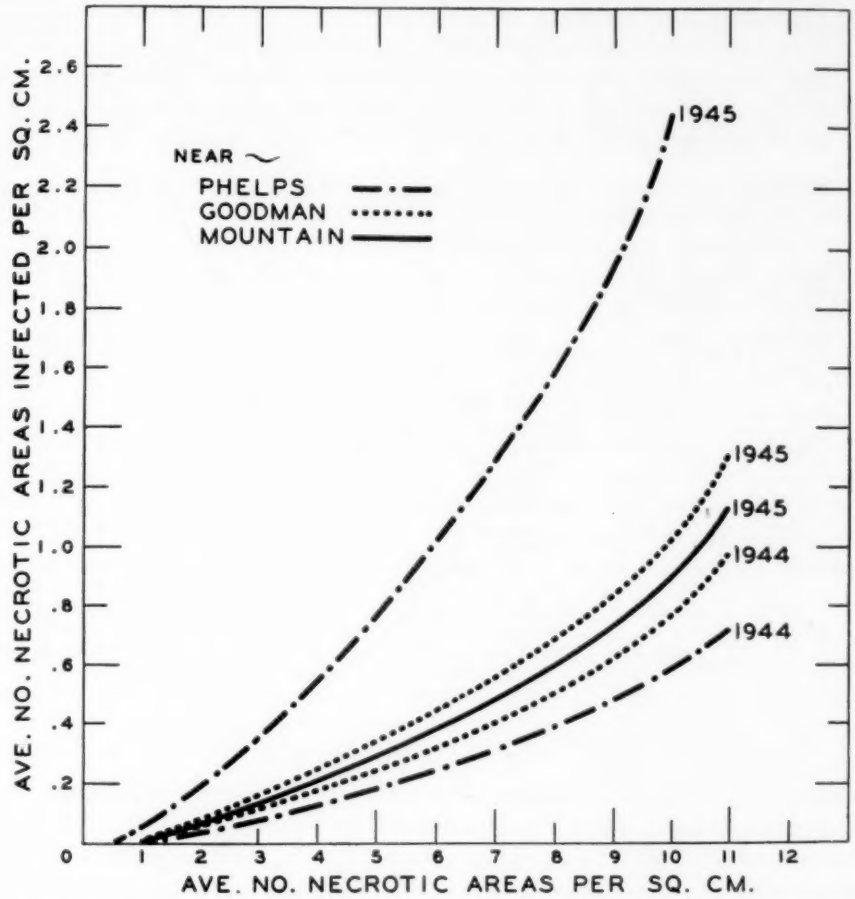

FIG. 2. Relation between the number of insect-initiated and necrotic cortical areas per square centimeter and the number of such areas containing *Chilonectria*.

insects did produce disease symptoms. A combination of heavy fungus infection introduced by spores and insects killed the trees rapidly.

In these tests attempts to determine how many insects were necessary to kill twigs by their feeding alone were unsuccessful, because the fungus could not be excluded.

TREE VIGOR AND BURN BLIGHT DEVELOPMENT

The effect of tree vigor on disease development was studied by weakening vigorously growing trees of similar size in various ways. In a trial begun

on August 3, 1944, the soil was removed and about one-half of the roots were cut from 5 trees. All dead twigs and branches were removed from these trees and from 5 nontreated controls. About 1 year later, the nontreated controls had a total of 49 inches of dead twig length caused by burn blight, an average of approximately 10 inches per tree. The root-pruned series, however, had a total of 198 inches of such dead twigs and branches, an average of approximately 40 inches per tree. This indicated that root-pruned trees were more susceptible to fungus attack.

Further evidence was secured from vigorous trees on which mechanical punctures were made into 45 uniform-sized branches. The needles were removed by hand from the 1-year-old wood and the area of the bark was

TABLE 3.—*Summary of puncture test on jack pine, July 12, 1945, to September 20, 1945, near Phelps, Wisconsin*

Mechanical punctures per square centimeter	Average diameter of necrotic areas[a]	Condition of twigs
No.	*Mm.*	
0	0	Foliage green and branches healthy.
5	1	Foliage green, small under-bark spots, not infected.
10	1 to 2	Foliage green, small under-bark spots, not infected.
15	2 to 3	Foliage green, small under-bark spots, 0.1 per cent of spots infected.
20	3 to 5	Foliage green, 5 per cent of spots infected.
25	3 to 10	Foliage green, about 50 per cent of the cortical area with infected spots coalescing and drying.
30	5 to 10	Foliage yellow, 50 to 75 per cent of cortical area infected and drying.
35	Girdled	Foliage yellow, 75 to 90 per cent of cortical area covered by coalescing infected areas. One of 5 branches dead.
40	Girdled	Two of 5 branches were dead with the fungus fruiting and 3 branches had 90 per cent of the cortex infected with coalescing spots. Three-quarters of the foliage was dead.

[a] No spores were artificially applied. The average is that from all 5 treated branches.

calculated. From 5 to 40 punctures per square centimeter were made with the multiple-pin block. Each treatment plus an unwounded control was replicated 5 times. The trial ran from July 12 to September 20, 1945. (See table 3.) The punctures were not inoculated artificially. However, *Chilonectria* was isolated from all branches in which the necrotic spots coalesced. From these results it appears that natural infection takes place through punctures and that widely scattered punctures are not so likely to become infected as those placed close together. This corresponds to the upturn in the curves of figure 2 showing more infections with an increase in number of insect-induced necrotic areas per square centimeter. This evidence, along with that from root-pruned trees, suggests that injury may increase susceptibility to natural infection by *Chilonectria*.

Since killing many twigs greatly reduced the functional needle-bearing area of a tree, the question arose as to whether this increased susceptibility

to fungus invasion. Some trials of drastic pruning were made during the summer of 1944. In one series with 10 replications approximately 0, 25, 50, 75, and 90 per cent of the needle-bearing areas were cut away. In another series with suitable controls and replicated 10 times in 4 locations in Wisconsin, about 80 per cent of the needle-bearing areas were cut off. By November, 1945, the severe pruning apparently had no effect upon natural disease development. If anything, the pruned trees showed less disease development than did the unpruned, although the difference was not statistically significant. All the trees, except one with 90 per cent of the foliage removed, survived. The new growth on the pruned trees was particularly vigorous.

The effect of various vigor-reducing treatments upon the development of disease symptoms following artificial inoculation was examined. The treatments were (1) none, (2) removal of all branches from the lower half of the tree, reducing the needle area by 60 to 80 per cent, (3) partial girdling of the main stem below the point of inoculation (last 3 inches of 1943 growth and first 3 inches of 1944 growth), and (4) root pruning to remove about 50 per cent of the roots. These trees were then inoculated in the current-year wood of the leaders with the hypodermic needle inoculator as follows: (1) control, distilled water; (2) a suspension of *Chilonectria* spores from the single-spore isolate No. 1; and (3) a suspension of *Chilonectria* spores from field-collected fruiting bodies. These treatments were replicated 5 times under each of the 4 types of pre-inoculation treatments. The series was established on August 23, 1944, in the Phelps, Wisconsin, area.

The inoculated trees turned yellow or brown above the point of inoculation and there was considerable cortical necrosis. By the next spring all the inoculated trees had dead tops above the point of inoculation. The tops of the control trees were still green and healthy.

Detailed examination of the trees was made during September, 1945, in the laboratory. Reisolations were attempted from every tree. The fungus was not isolated from the control trees and was recovered from all inoculated trees.

Spread of the fungus in infected trees was also studied. The cortex in the diseased region was removed and the advance down the stem from the lowest needle puncture was measured and the results averaged for treatments and for source of inoculum. The averages are presented in figure 3. Although the fungus advanced to some extent on vigorous trees, it moved considerably farther on weakened trees. This provides further evidence that tree vigor influences burn blight development, and suggests that site factors might influence susceptibility.

Some indications of the effect of site quality were observed on two plots, one poor and the other good. But no effort was made to isolate the effect of each factor, such as soil type and fertility, drainage, exposure, moisture, slope, ground cover, overstory, and others. The study areas were both on

a light sandy loam soil and were only about ¾ mile apart. On the poor site the soil moisture content (dry weight basis) at a 6-inch depth was 6.1 per cent at a time when it was 11.7 per cent on the good site. The soil reaction in both plots was pH 5.5. The average length of 25 growing tips recorded on June 15, 1945, was 2.3 inches for the poor site and 4.1 inches for the good site.

Disease development was observed on these plots from June, 1944, to November, 1945. The records showed that there was little top dying on the good site while all 25 trees on the poor site had died down from the top at least one-fourth the length of the main stem. The total length of dead twigs and branches was measured and the records showed the dead lengths to be 3 feet on the good site and 423 feet on the poor site. Because the insects

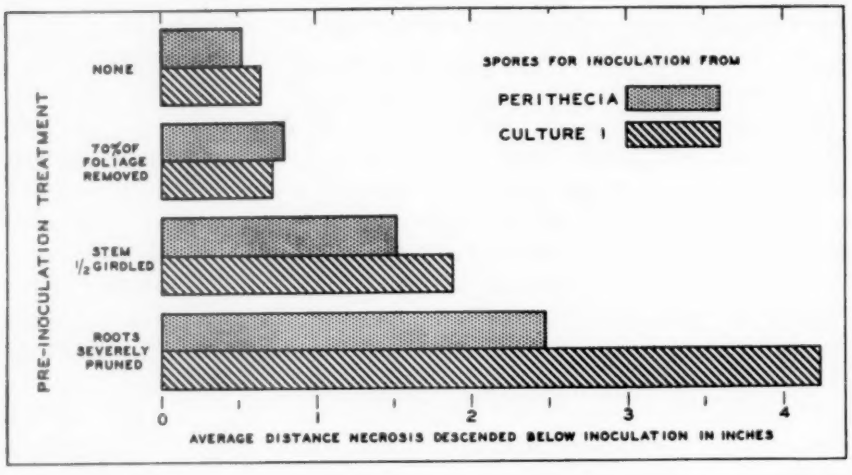

FIG. 3. Relation of pre-inoculation weakening (average for 5 trees) to the rate of fungus advance down the main stem of jack pine.

may have inoculated the trees on the poor site more than those on the good site, the reaction to artificial inoculations was examined.

Puncture inoculations were made with a spore suspension from single-spore culture No. 1 on 5 jack pine stems in each plot. The records taken 14 months later, in September, 1945, showed that the top of every tree had been killed in each area. However, on the good site the fungus had advanced down the stem an average of only 1.3 cm. below the lowest inoculation wound, while on the poor site the advance averaged 10.9 cm. These observations suggested that the site and the resulting condition of the trees influenced the natural incidence as well as the artificially induced development of disease.

SEASONAL DEVELOPMENT

Periodic observations were made on the seasonal development of burn blight on jack pine on various plots in northeastern Wisconsin. The results

are shown in figure 4. In A, trees showing top dying were counted. In B and C the amount of top dying was measured and recorded in percentage of total heights. Most of the trees were from 6 to 14 feet high. In D the dead twigs and branches were removed and their total length was measured. In E the advancing margin down the main stem was determined by gently removing the dead bark scales and by observing the most advanced brown area. This was marked with paint, a different color for successive readings.

Although the total readings gave a clear picture of the extent of the damage, they failed to show the critical time of year for the most rapid development. Consequently the same data were converted to the change per week. Such records appear opposite the others in figure 4.

The total number of trees with top dying (Fig. 4, A) increased from July 2, 1944, to September 13, 1945. The conversion of these data to an increase per week basis (A′) shows that the rate of increase was not constant. There were two peaks, one on August 21, 1944, and the other on July 16, 1945, with each peak followed by a sharp decline. The increases were chiefly during the warm moist spring and early summer weather. This is the time when plants are apt to be susceptible. This is likewise ahead of the time when the spittle insects feed on pine. In 1945 the insects were later than usual.

Twenty-nine per cent of the main stems were killed on 145 trees in 1945 in the Phelps area. The increase in downward progress of top dying (Fig. 4, B′) was at its peak, 23 per cent per tree per week, on July 16, and declined thereafter. Again the decline in rate of dying was during the period of greatest spittle insect activity. The increase in rate of dying was from April to July. The results of trials on 10-tree plots located near Goodman, Mountain, Phelps, and Stiles, Wis., (Fig. 4, C) agreed closely with those on the 145 trees in the Phelps area (Fig. 4, B). This indicates that the situation at Phelps was not due to local conditions.

Figure 4, D shows a progressive increase in incidence of dead twigs and branches based on foliage symptoms. However, as seen before, the weekly rates of increase (Fig. 4, D′) hit peaks in midsummer and declined rapidly in 1945 during the period of spittle insect activity.

The average advance of the necrotic margin showed an increase from October, 1944 to October, 1945 (Fig. 4, E). The rate of weekly increase (Fig. 4, E′) showed declines in late summer, which were in accord with the observations on disease manifestations previously described.

A summary of the seasonal development data, as presented in figure 4, indicates that the most rapid disease development occurred during the spring and early summer. The greatest fungus activity was during the same period. This is most easily explained by favorable environmental conditions for the growth of the fungus introduced by a heavy fungus inoculation from the insect vector the previous year.

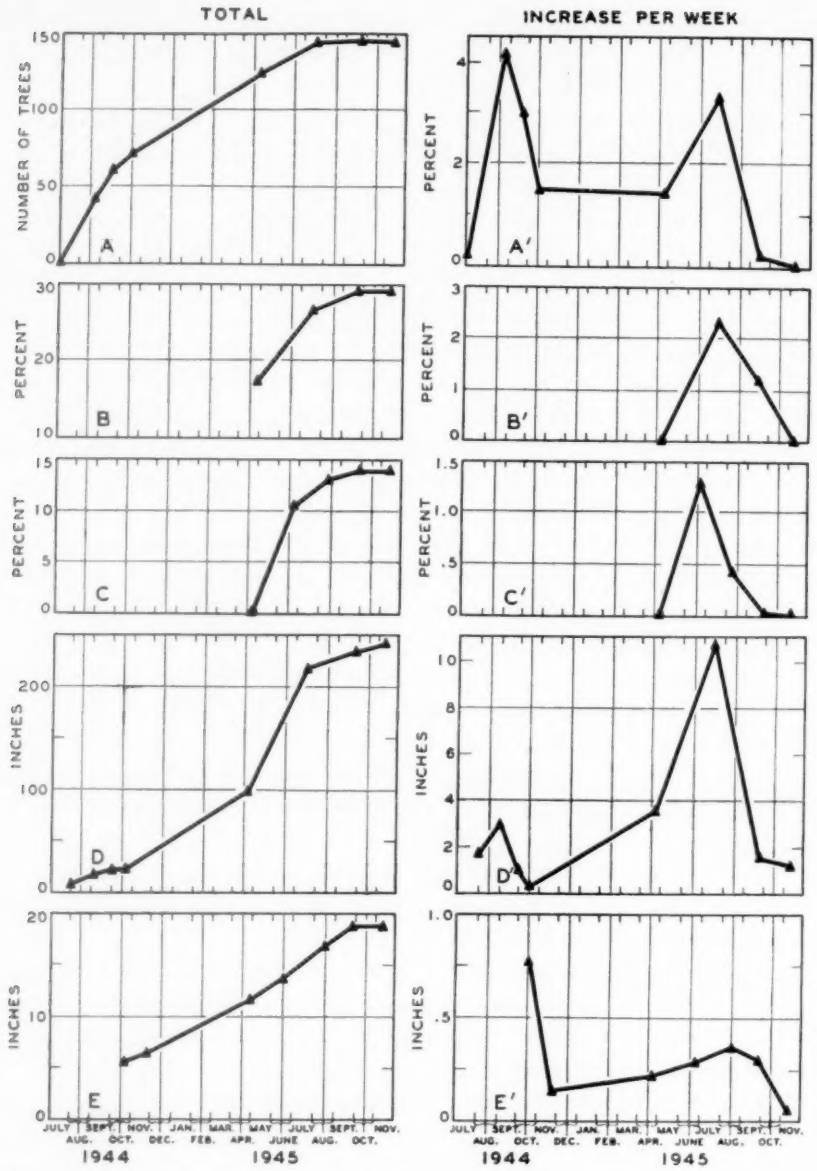

FIG. 4. Summary of disease development on jack pine. A. Number of trees dying near Phelps (6 plots of 1/40 acre each). B. Amount of top dying in percentage of total height (average of 145 trees) as in A. C. Same as B except on 10 trees each near Goodman, Mountain, Phelps, and Stiles. D. Total length of dead twigs and branches near Phelps (average of 13 trees). E. Total necrotic margin advance on main stem, near Goodman, Mountain, and Phelps (average of 214 trees). A′, B′, C′, D′, and E′ same as A, B, C, D, and E, respectively, except the line in A′ is in terms of increase per week, B′, C′, D′, and E′ in terms of increase per tree per week.

DISCUSSION

From the evidence presented it appears that the fungus *Chilonectria cucurbitula* associated with burn blight of jack and red pines is pathogenic but is dependent upon some injury, such as the punctures made by the spittle bugs, for entry. The spittle bugs fed on the pines in July, August, and September, inoculating the trees with *C. cucurbitula*. This fungus then killed twigs, branches, and even main stems. It has appeared most active during warm spring weather.

Although *Chilonectria*, if introduced through some other injury, can induce disease independently of the spittle insect, there are indications that the insect alone also can cause serious damage. In any case the two agencies operating together present a serious problem for jack and red pine.

Looking to the future, there appear several possibilities worth considering in relation to control measures. They include: (1) the control of the spittle insect by one or another of the recently developed and powerful insecticides; (2) the selection of sites where trees grow vigorously and, correspondingly, where they may be perhaps less attractive to the spittle insect and less susceptible to the *Chilonectria* than they seem to be on poor sites; and (3) for new plantings it seems desirable to arrange for suitable interplanting with other species or at least for trees in different age classes. Any procedure deserves attention if it tends to reduce the accumulation of large populations either of the spittle insect or of *C. cucurbitula*.

SUMMARY

Jack pine trees affected with burn blight have been dying since 1941 in northeastern Wisconsin and in Michigan. In 1945 there were 64 known disease centers in 9 northeastern Wisconsin counties.

Small twigs at the tops of the trees turn yellow and then brown, and the disease continues downward in the branches and main stems.

Examination revealed that feeding punctures of *Aphrophora saratogensis* Fitch were common on the diseased trees and that necrotic spots caused by *Chilonectria cucurbitula* (Curr.) Sacc. developed around some of the punctures. These brown spots usually enlarged, especially during the warm weather of the following spring and summer, and coalesced, girdling the twigs. Perithecia often developed 4 weeks after inoculation. The fungus moved down the cortex of 10-feet high susceptible trees and killed them in 1 to 3 years.

Chilonectria cucurbitula was isolated, identified, inoculated into trees with positive results, and reisolated. Sixteen different isolates were used in inoculating 412 trees in northern Wisconsin and in the greenhouse or field at Madison.

The adult spittle insect was present from early July until frost. Its feeding punctures appeared commonly on twigs examined in the infected areas. *Chilonectria* was found on every one of 85 adults examined by cul-

tural technique. When adults were placed in a cage, the disease developed on twigs they attacked. Likewise, infection frequently developed about feeding punctures in nature. The evidence suggests that these adults not only acted as vectors but also as agents to weaken the twigs more or less and to provide the *Chilonectria* with a favorable entry.

Vigorously growing twigs, branches, and trees seemed to be relatively resistant and poorly growing trees to be relatively susceptible both to insect inoculation and to puncture inoculation with a spore suspension.

During the growing season feeding by the Saratoga spittle insect occurred in July and August and to a less extent in September. Most of the twig dying appeared with the advent of warm spring and summer weather after the fungus had incubated during the late summer, fall, and early spring.

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